Vol. XXIII September, 1946 No. 5

PUBLIC LIBRARY
OCT 1 1946

CEREAL CHEMISTRY

Published bimonthly by the American Association of Cereal Chemists
at Prince and Lemon Sts., Lancaster, Pa.

W. F. Geddes, Editor in Chief
Kathleen Webb, Assistant Editor
Donald E. Smith, Managing Editor
University Farm, St. Paul 1, Minnesota

Editorial Board:
J. A. Anderson, J. S. Andrews, M. J. Blish, G. Garnatz,
Betty Sullivan, C. O. Swanson

CONTENTS

Page

Grain Storage Studies. IV. Biological and Chemical Factors Involved in the Spontaneous Heating of Soybeans. *Max Milner and W. F. Geddes* 449

The Effect of Pressure on Macaroni Discs: Size and Number of Air Bubbles in Relation to Light Transmission. *Glenn S. Smith, R. H. Harris, Ethel Jesperson, and L. D. Sibbitt* 471

A Micro-Milling Technique Using the Hobart Grinder. *Karl F. Finney and William T. Yamazaki* 484

A Study of Sorghum and Sorghum Starches. *F. E. Horan and Marie F. Heider* 492

Composition of the Component Parts of the Corn Kernel. *F. R. Earle, J. J. Curtis, and J. E. Hubbard* 504

Influence of Ingredients on Thiamine Retention and Quality in Baking Powder Biscuits. *Alice M. Briant and Marcia R. Hutchins* 512

Report of the 1945–46 Methods of Analysis Subcommittee on Thiamine Assay. *Aaron Arnold* 521

Book Reviews 527

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $6 per year. Foreign postage, 50 cents extra. Single copies, $1.25; foreign, $1.35.

Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the act of August 24, 1912.

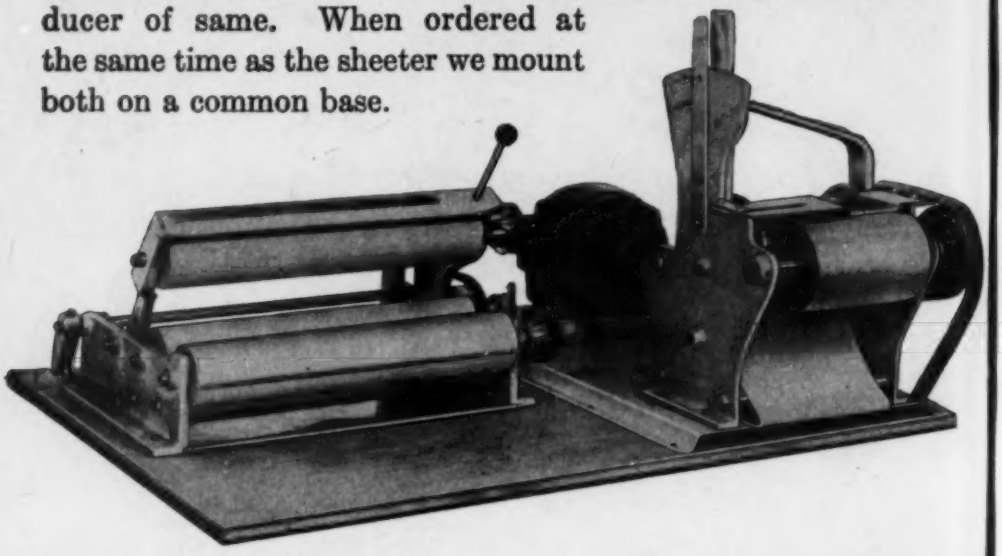
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized February 16, 1924.

SHEETING ROLLS

for the TEST BAKING LABORATORY

We manufacture two sizes of sheeting rolls; the 3″ width for Pup Loaves and the 6″ width or 1 lb. size for all loaves from Pup to Standard loaves, which model is shown below mounted with three-roll-type moulder.

Our Three-Roll-Type Moulder can be attached to either size sheeting roll, and is driven from power take-off of speed reducer of same. When ordered at the same time as the sheeter we mount both on a common base.



UNIVERSAL "THREE-ROLL-TYPE" MOULDER

PRICE $80.00

Test Bake Moulding can be done mechanically with the operator confident that his doughs are:

1. Moulded and sealed in an effective and GENTLE manner,
2. Ends of doughs are not abused and
3. Entire operation on dough is visible at all times.
4. Variation between operators is reduced.

1 LB. SIZE POWER SHEETING ROLL

as shown above, including G.E. 110 V., A.C. motor, speed reducer and foot switch.

PRICE $160.00

PUP SIZE POWER SHEETING ROLL

Same as above but 3″ width

PRICE $125.00

CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY

LINCOLN, NEBRASKA

EXTRA YARDAGE WITH THE WIND!

Vitamin enrichment adds sales yardage to your product

In golf, the wind at your back adds yards to your drive. So vitamin enrichment is adding yardage to bread sales. Continue to feature the word "ENRICHED" on your wrapper.

In a recent survey*, 77.4% of the American housewives questioned emphasized that they prefer foods which have been enriched with vitamins; 99% stated that they consider vitamins of great importance in the food they serve their families.

Remember, the mechanics of bread enrichment are simple with 'ROCHE' *SQUARE* ENRICHMENT WAFERS, distributed by yeast companies.

*Fawcett Women's Group

Vitamin Division

ROCHE

HOFFMANN-LA ROCHE, INC.

Roche Park, Nutley 10, New Jersey

We are justifiably proud of the evergrowing list of

FARINOGRAPH *users:*

Over the past few years we have been able to add the following names to this list:

American Flours, Inc.
Newton, Kan.

American Institute of Baking
Chicago, Ill.

Anheuser-Busch, Inc.
St. Louis, Mo.

H. C. Cole Milling Company
Chester, Ill.

Colorado Mlg. & Elev. Co.
Denver, Colo.

Consolidated Flour Mills
Wichita, Kan.

Doty Technical Laboratories
Kansas City, Mo.

Valier & Spies Milling Co.
St. Louis, Mo.

Kansas Flour Mills Company
North Kansas City, Mo.

Higginsville Flour Mill
Higginsville, Mo.

Hunter Milling Company
Wellington, Kan.

Inland Mills, Inc.
Des Moines, Iowa

International Milling Co.
Detroit, Mich.

William Kelly Milling Co.
Hutchinson, Kan.

Kelly-Erickson Company
Omaha, Neb.

Langendorf United Bakeries
San Francisco, Cal.

Joe Lowe Corporation
New York City

Joe Lowe Corporation
Chicago, Ill.

Maney Milling Company
Omaha, Neb.

Mennel Milling Company
Toledo, Ohio

Midland Flour Milling Co.
Kansas City, Mo.

Montana Flour Mills Co.
Great Falls, Mont.

National Biscuit Company
Cheney, Wash.

National Tea Company
Chicago, Ill.

New Era Milling Company
Arkansas City, Kan.

Omar, Inc.
Omaha, Neb.

C. J. Patterson Company
Kansas City, Mo.

Pillsbury Mills Company (2nd Inst.)
Minneapolis, Minn.

Quality Bakers of America
New York City

Rodney Milling Company (2nd Inst.)
Kansas City, Mo.

Russell-Miller Milling Company
Alton, Ill.

Russell-Miller Milling Company (2nd Inst.)
Minneapolis, Minn.

F. W. Stock & Sons, Inc.
Hillsdale, Mich.

Vanderbilt Foods Corporation
East Norwalk, Conn.

Wallace & Tiernan Company, Inc.
Newark, N. J.

Walnut Creek Milling Company
Great Bend, Kansas

Ward Baking Company
New York City

Washburn Crosby Div.
General Mills, Inc.
Kansas City, Mo.

Williams Bros. Company
Kent, Ohio

●

You, too, can have one of the latest model FARINOGRAPHS. We have an attractive rental proposition so that you may try the instrument thoroughly before purchase. Write us for details.

●

BRABENDER CORPORATION **Rochelle Park, N. J.**

TELL US MORE, Teacher!

● Maybe you're like a lot of food technologists and salt buyers who have gone along thinking all salt is the same. Actually, various brands and grades and grains of salt differ in many respects.

Take solubility: In salting cheese, slow solubility of salt is highly important and desirable. For otherwise, salt is lost in the whey. On the other hand, in salting butter, salt must dissolve with lightning speed. If the butterfat is on the soft side at certain seasons, butter

salt must dissolve so quickly that over-working is avoided. Otherwise, the butter is likely to become mottled or marbled—may lose its moisture, become leaky. Yet, if the salt is not properly dissolved, the butter may be gritty.

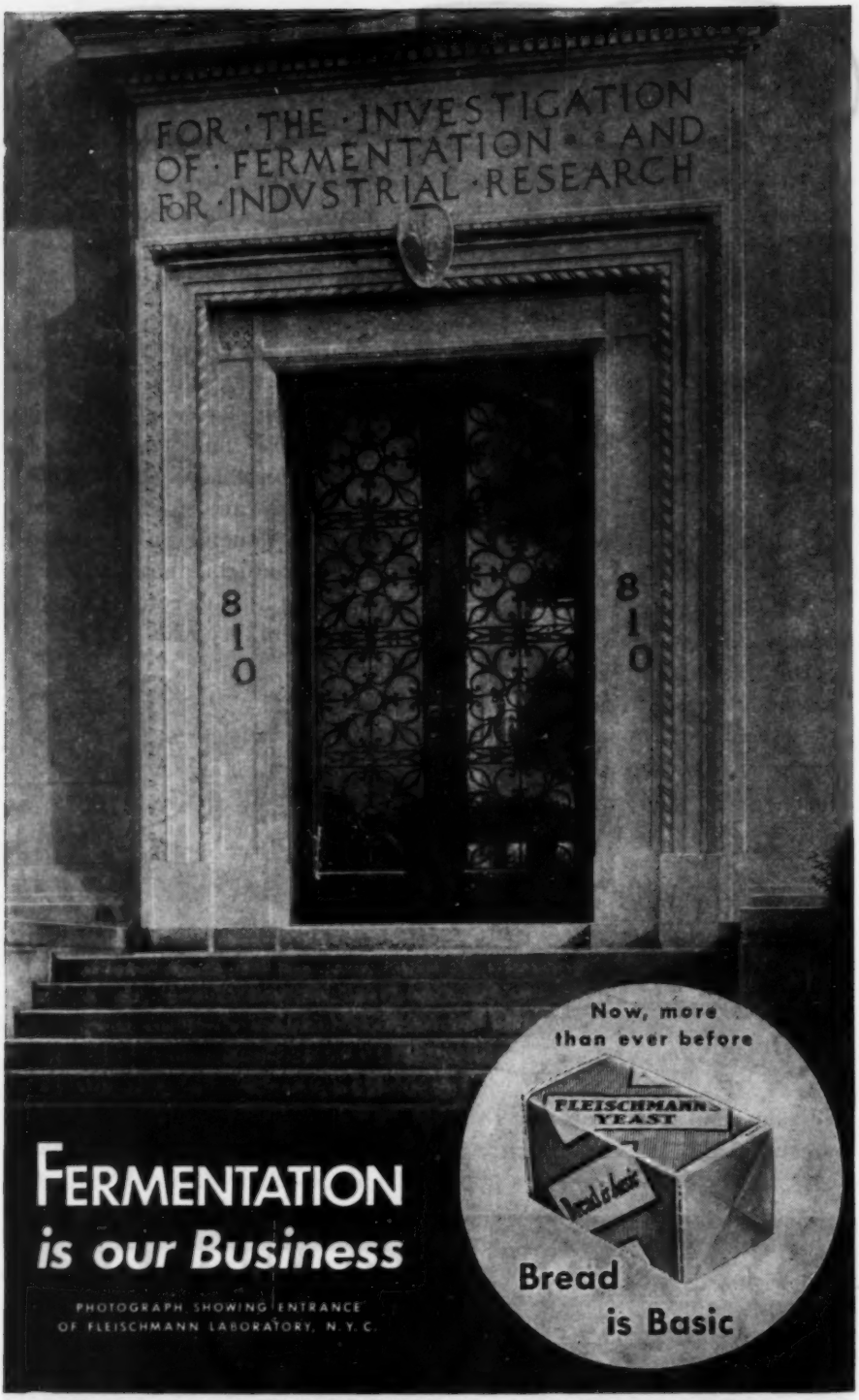
So we have definite solubility standards for Diamond Crystal Salt. Our Butter Salt, for example, dissolves completely in water at 65° F. in less than 9.8 seconds—average rate, 9.2 seconds. That is why so many quality-minded food processors depend on Diamond Crystal products, manufactured under strict quality-control standards for solubility rate.

WANT FREE INFORMATION ON SALT? WRITE US!

If salt solubility enters into your processing, write our Technical Director. He will recommend the correct Diamond Crystal Salt for best results. Diamond Crystal, Dept. M-12, St. Clair, Michigan.

DIAMOND CRYSTAL SALT

ALBERGER PROCESS



BACK YOUR FUTURE WITH U. S. SAVINGS BONDS

THESE 2 POWERFUL DOW FUMIGANTS

answer practically every food fumigation problem

DOW Chloropicrin

This well-known fumigant is especially well adapted for use in buildings not sufficiently well constructed to confine other gases. It is also widely used for rodent control, since very small quantities properly placed, will dispose of them in a hurry. Where "spot" fumigations are desired because of localized infestation, Dow Chloropicrin is thoroughly reliable.

THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN

New York • Boston • Philadelphia • Washington • Cleveland
Detroit • Chicago • St. Louis • Houston • San Francisco
Los Angeles • Seattle

Because of its penetrating power —unmatched by any similar product—Dow Methyl Bromide reaches the center of 140-lb. bags of grain stacked in large piles. It quickly accomplishes a complete kill of rodents and insects in all stages of development and, because it vents rapidly, permits a return to work the following morning.

DOW Methyl Bromide

Additional information on these two powerful answers to fumigation problems is available on request, or Dow can put you in touch with a competent industrial fumigator specializing in the food field.

DOW

CHEMICALS
INDISPENSABLE TO INDUSTRY

MADE FOR EXACTING USES

COVO Shortenings are all-vegetable, all-hydrogenated, always uniform, always dependable. All are made to specifications for exacting uses. There is one best suited to your particular needs.

Covo

—the outstanding all-purpose shortening for fine cakes and icings, cookies and piecrust. Specially refined for heat endurance in frying.

Covo "S. S."

—the special shortening for prepared mixes—for biscuit and cracker manufacture, and for all other products requiring extra stability for long shelf life.

Covo Super-Mix

—the modern emulsifier type shortening that holds extra liquids, giving exceptionally fine eating quality and extended freshness to all cakes, icings, sweet yeast doughs.

Covo Shortenings

LEVER BROTHERS COMPANY • General Offices: Cambridge 39, Mass.

Invitation to Cereal Chemists

Your inquiries on food and cereal chemistry are welcomed by Monsanto research laboratories. You may find this service particularly gratifying — especially if you are interested in food-grade phosphoric acid and phosphates derived from Monsanto-made elemental phosphorous of better than 99.9% purity.

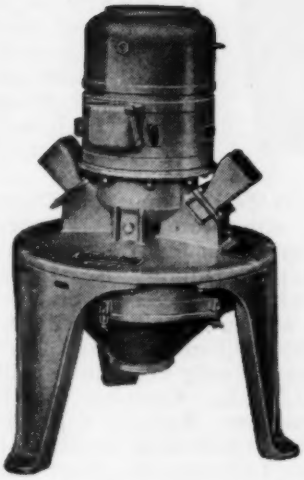
No obligation is involved — technical information and samples are gladly furnished. Please contact the nearest Monsanto Office, or write: MONSANTO CHEMICAL COMPANY PHOSPHATE DIVISION, 1700 South Second Street, St. Louis 4, Missouri. District Offices: New York, Chicago, Boston, Detroit, Charlotte, Birmingham, Los Angeles, San Francisco, Seattle, Montreal, Toronto.

MONSANTO CHEMICALS
SERVING INDUSTRY . . . WHICH SERVES MANKIND

PROTECT YOUR PRODUCTS

"ENTOLETER" SYSTEM GIVES CONTINUOUS

BUG CONTROL plus!



1 **KILLS ALL INSECT LIFE**

Now, more than ever, your plant and products need the "ENTOLETER" system of continuous insect control. It helps you to avoid waste of scarce food materials . . . to protect your flour . . . to insure the high standards of your products.

2 **ESSENTIAL IN FRAGMENT CONTROL**

The "ENTOLETER" system is being used successfully to reduce fragment count. Send for copy of our latest bulletin describing methods of application.

3 **AIDS PRODUCT UNIFORMITY**

Food plants and flour mills have found that the "ENTOLETER" system offers an excellent means of blending flours, mixing ingredients in compounds and improving vitamin distribution. This, alone, more than justifies its cost.

ENTOLETER
INFESTATION DESTROYER

CONTINUOUS INSECT CONTROL SYSTEM

ENTOLETER DIVISION
The Safety Car Heating and Lighting Co., Inc.
1153 Dixwell Ave., New Haven 4, Conn.

Send full information on "ENTOLETER" Continuous Insect Control System.

Name

Company

Address

City, Zone and State

Available NOW!

COLEMAN pH ELECTROMETER

MODEL 3

A limited number of these famous reliable pH electrometers are now available.

STOCK DELIVERY

ACCURATE to within the limits of the glass electrode, namely .05 pH. Intended for the laboratory, hundreds have also been purchased for plant control.

Only the Coleman design eliminates glass electrode breakage. The electrodes are sealed and filled at the factory and safely mounted to avoid all contact with the sample cup. The KCl junction is easily and thoroughly flushed . . . no ground joints to carry over from test to test! Chassis is sealed and moisture cannot cause leakage errors. Only attention required is replacement of batteries, tubes ond glass electrode . . . the latter is guaranteed for six months and usually lasts over a year!

No. 3537-A Coleman pH Electrometer for pH only. Single scale, 0-13 pH by 0.1 divisions. Easily read to pH by 0.02 pH and amply sensitive to same limits. Electrical accuracy 0.1%. Electrochemical accuracy 0.05 pH. Complete with Sealed Ag-AgCl Glass and Reference Electrodes, buffer solution, tubes, batteries. Each $180.00

No. 3538-D Coleman pH Electrometer with Automatic Temperature Compensator for oxidation reduction and pH readings. Scale changing switch allows direct reading 0-13 pH and also in millivolts over the range 0-1300 millivolts by 10 millivolt divisions. Easily read to 2 millivolts or 0.02 pH. Complete with Sealed Glass and Reference Electrodes, Redox Electrodes, buffer solution, tubes, batteries, etc. Each . . $235.00

pH BUFFER TABLETS

More reliable than liquid buffers. 12 in glass vial, $2.00. This means that 10 100 ml of solution costs only 16.7c! Write for Bulletin or order from this advertisement.

Available in steps of 0.2 pH from 2.0 to 12.00 pH.

Write for catalog CC9

WILKENS-ANDERSON CO.

111 NORTH CANAL STREET • CHICAGO 6, ILLINOIS

PURE VITAMINS
—Products of Merck Research

Thiamine Hydrochloride U.S.P.
(Vitamin B_1 Hydrochloride)

Riboflavin U.S.P.
(Vitamin B_2)

Niacin
(Nicotinic Acid U.S.P.)

Niacinamide
(Nicotinamide U.S.P.)

Pyridoxine Hydrochloride
(Vitamin B_6 Hydrochloride)

Calcium Pantothenate
Dextrorotatory

Ascorbic Acid U.S.P.
(Vitamin C)

Vitamin K_1
(2-Methyl-3-Phytyl-1,4-Naphthoquinone)

Menadione U.S.P.
(2-Methyl-1,4-Naphthoquinone)
(Vitamin K Active)

Alpha-Tocopherol
(Vitamin E)

Alpha-Tocopherol Acetate

Biotin

Merck & Co., Inc. now manufactures all the vitamins commercially available in pure form, with the exception of vitamins A and D.

Merck research has been directly responsible for many important contributions to the synthesis, development, and large-scale production of individual vitamin factors in pure form.

In a number of instances, the pure vitamins may be considered to be products of Merck research. Several were originally synthesized in The Merck Research Laboratories, and others have been synthesized by Merck chemists and collaborators in associated laboratories.

Because most of the known vitamins have now been made available in pure form, effective therapy of specific vitamin deficiencies can be conducted on a rational and controlled basis, under the direction of the physician.

MERCK VITAMINS

MERCK & CO., Inc. RAHWAY, NEW JERSEY

Manufacturing Chemists

MERCK MERCK

Better

KJELDAHL NITROGEN APPARATUS

Unparalleled system of fume disposal (Patented)
Not available in any other apparatus

Your Choice of Many Arrangements

Only One Choice of Efficiency and Durability

Pictured—Combination Digestion and Distillation Unit, Electrically Equipped and with 3 heat switches.

Detailed specifications and full information will be sent on request without any obligation on your part.

Also

LABORATORY EQUIPMENT TABLES

"GOLDFISCH" ELECTRIC HEATERS

"GOLDFISCH" EXTRACTION APPARATUS

CRUDE FIBRE APPARATUS

"LABCONCO" WIDE RANGE GAS BURNERS

Catalogue on Request

Your inquiry is invited. No trouble to submit proposals on your requirements and, of course, without obligation to you.

Manufactured and sold direct to the user by

Laboratory Construction Company

1113-1115 Holmes Street, Kansas City, Missouri, U. S. A.

CEREAL CHEMISTRY

VOL. XXIII SEPTEMBER, 1946 No. 5

GRAIN STORAGE STUDIES. IV. BIOLOGICAL AND CHEMICAL FACTORS INVOLVED IN THE SPONTANEOUS HEATING OF SOYBEANS [1]

MAX MILNER [2] and W. F. GEDDES [3]

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota

(Received for publication May 21, 1946)

Observations on the spontaneous heating of grain have usually been made with seeds stored in bulk. Until the work of Ramstad and Geddes (1942) and of Sallans, Sinclair, and Larmour (1944) who carried out heating studies under virtually adiabatic conditions, the few laboratory studies which have been reported have utilized either poorly insulated lots of grain or Dewar flasks.

The fundamental causes of the spontaneous heating of grain have not been clearly elucidated. The conclusion of Bailey and Gurjar (1918) and Bailey (1940) that the heating of stored wheat and other grains is due to seed embryo respiration has been questioned by several investigators who have pointed out that molds resident on normal grain may proliferate at storage moisture levels, and that their metabolic and respiratory activities are strongly thermogenic. Cohn (1890) early found that germinating seeds maintained in an insulated box would reach temperatures of only 40°C due to the thermogenic effects of seed respiration under germinating conditions. At this temperature the seedlings were killed. He attributed subsequent temperature increases to 65°C to thermophilic microorganisms. Darsie, Elliot, and Peirce (1914) carried out similar studies in Dewar flasks and pointed out that temperature increases due to the respiration of germinating seeds were slight compared to those caused by mold growth. Gilman and Barron (1930) concluded that "in bins of stored grain, marked increases in temperature may be ascribed to mold growth."

[1] Paper No. 2304, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, March, 1945.

[2] Research Associate, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

[3] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

Recent literature on the thermogenic activity of microflora growing on a variety of moist agricultural materials has not been adequately related to spontaneous heating phenomena in stored grain. Among such studies are those of James (1927), James, Rettger, and Thom (1928), Bakke and Henson (1933), Bakke and Noecker (1933), Harrison (1934), Carlyle (1940), Wedberg and Rettger (1941), Norman, Richards, and Carlyle (1941), and Carlyle and Norman (1941). Browne (1929, 1933), Haldane and Makgill (1934), and Hoffman (1935, 1940) have speculated on the relationship of microfloral heating to spontaneous combustion in biological materials. Most of the studies cited were carried out at high moisture values (usually about 30%) where numerous types of molds, yeasts, and bacteria proliferated. They have indicated that two ranges of temperature exist due to microfloral heating. The first range, attributed to the metabolic and respiratory activity of mesophilic nonspore formers, in which class the molds are included, ends at about 50°–55°C, while a secondary heating to a maximum of 70°C is due to thermophilic bacteria. Milner and Geddes (1946) have emphasized that only certain mold species, namely those which will proliferate at relatively low humidities, are important in the respiratory activity of stored grain. Several writers have indicated that bacterial growth is not to be expected on natural products at humidity levels below 95%.

With a view to obtaining more precise information on the nature and course of the spontaneous heating of soybeans than was available, the adiabatically controlled apparatus of Ramstad and Geddes (1942) was refined and its utility extended by the addition of apparatus for the control of aeration of the heating samples, in such a manner that the respiratory characteristics could be precisely followed. In addition to studies on the spontaneous heating of normal soybeans at moisture levels favoring storage deterioration, adiabatic trials were carried out with pure cultures of microflora isolated from soybeans and inoculated on sterile seeds.

Apparatus

Two adiabatically controlled respirometer units, schematically represented in Figure 1, were used. The insulated air bath *A* consisted of a wooden thermostat covered with two layers of celotex. Dewar flask *D*, which may be one quart, one pint, or half-pint in size, contains the seeds whose heating characteristics are to be studied. Copper-constantan thermopile *T* has 24 junctions, 12 in the seed sample and 12 exposed on the outside of the Dewar flask to the air of the bath. Slight heating of the seeds in *D*, causing a temperature differential to exist between the two ends of the thermopile, results in

a flow of electric current through the coil of a sensitive D'Arsonval galvanometer *G* to which a mirror is attached, which in turn rotates a reflected light beam from a fixed integral light source along path *X* to a photoelectric cell actuating a relay in box *R*, which controls heater coils (*H*) in the air bath. The air in the thermostat is circulated continuously over the heaters by fan *S* and motor *M* through holes in the baffle *B*. When the air temperature of the bath reaches that of the seeds in the Dewar flask, the current in the thermopile circuit falls to

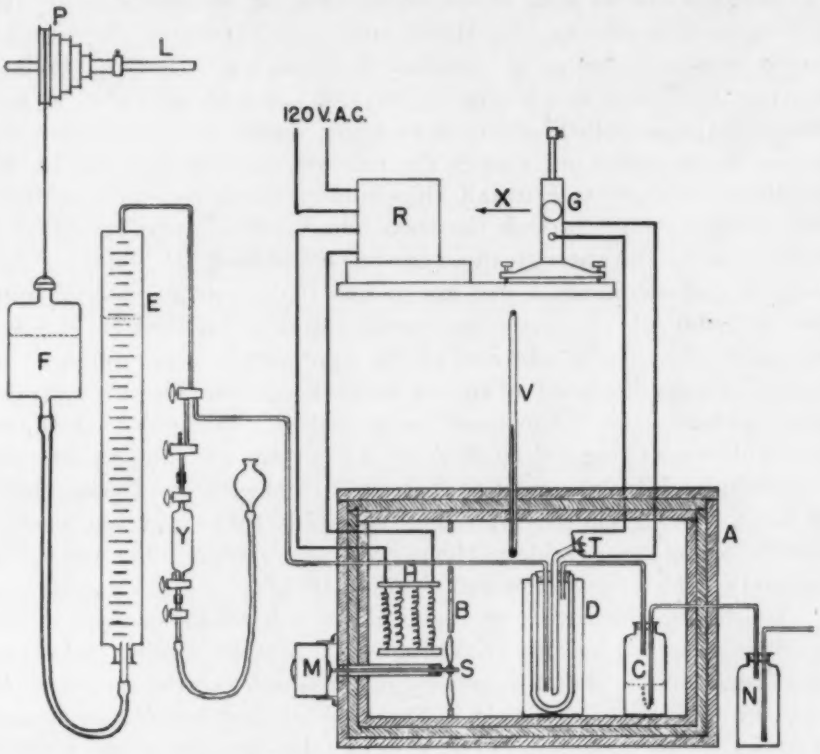


Fig. 1. Diagram of apparatus used to study the spontaneous heating and respiration of seeds.

zero and the torsion of the galvanometer coil suspension causes the light beam to recede from the photocell, thus interrupting the current to the heaters. The temperature of the air bath is thus continuously in step with that of the seeds in the Dewar flask, which are thus allowed to heat under virtually adiabatic conditions. A continuous curve of seed heating may be obtained over periods of days or weeks by simply reading the thermometer *V* inserted in the air bath at regular intervals. Not shown in the diagram is an auxiliary heater controlled by an external rheostat, which is used to compensate for heat

losses from the system as the temperature of the apparatus rises. In practice, the apparatus is initially calibrated by placing warm water (45°–60°C) in the Dewar flask and adjusting the galvanometer suspension torsion until the bath actually cools about 0.2°C in 24 hours. Conditions are thus not strictly adiabatic, but any possibility of the seeds being heated by the system in this temperature range is thereby avoided. At the end of a heating trial with seeds, the apparatus is again checked with warm water in the same manner.

Aeration of the seeds in the Dewar flask is accomplished by the same system as described by Milner and Geddes (1945) in their studies on soybean respiration at constant temperature. Leveling bulb *F* moving downward slowly due to the slow rotation of pulley *P* and lineshaft *L* (controlled by a reduction gear motor and chain drive not shown in the diagram) causes the calcium chloride solution in the graduated spirometer *E* to fall, thus drawing outside air at a continuous, constant rate through the soda lime bottle *N*, the humidifying solution in *C*, and through the seeds in Dewar flask *D*. Pulley *P* has steps of various diameter, making possible daily aeration rates of from 250 to 2,000 ml. Entering air streams were humidified by passage through sulfuric acid solutions of the appropriate vapor pressure to yield a relative humidity of the air which was in equilibrium with the seed moisture value (Milner and Geddes, 1945). Samples of the daily accumulation of respired air in *E* are withdrawn for analysis into gas sample tube *Y* by means of the stopcocks and mercury leveling bulb shown. The air samples were analyzed daily for oxygen and carbon dioxide using the Haldane-Henderson gas analyzer following the method outlined by Milner and Geddes (1945).

To obtain information on chemical and microfloral changes in the heating seeds as a heating trial progressed, a number of small bottles (not shown in the sketch), containing subsamples of the seed used in the Dewar flask, were placed in the air bath and aerated by means of an independent siphon bottle system. By this procedure, control of the temperature in the air bath was accomplished by the seeds heating in the Dewar flask, while small samples of the same grain, subjected to the same temperature changes, could be removed periodically for chemical and microfloral examination.

Anomalous Behavior of Thermopile-Galvanometer Controlled Adiabatic Thermostats. During the course of the present studies, it was noticed that calibration of the adiabatic thermostats with warm water to maintain either constant temperature or a slight cooling rate, at any given initial temperature, did not yield the same cooling rate when water of a different temperature was used. Thus, for example, if the instrument were adjusted at 25°C to cool approximately 0.1°C per 24

hours, the same calibration at 50°C would yield a cooling rate of about 0.5°C per 24 hours, in spite of the fact that at both temperatures the fixed heaters in the thermostat maintained the temperature of the air to within a few degrees of that of the water in the Dewar bottle. Conversely, a small cooling rate adjustment at 50°C (0.1° to 0.2°C per 24 hours) would result in slight ***heating*** of the system at 25°C in the order of 0.2° to 0.4°C in 24 hours. Attempts to overcome this difficulty were unsuccessful.

Among the factors investigated which might have a bearing on this phenomenon were: (1) thermal-electric effect in thermopiles, which is manifested by a nonlinear response of the thermopile current to temperature; (2) heat conduction along thermopile leads; (3) change of resistance and current of thermopile circuit with temperature; and (4) radiant heating effects in the system. None of these factors appeared to be involved in this anomalous behavior.

This phenomenon did not nullify the value of the adiabatic technique, inasmuch as its effects were recognized. Generally, the adiabatic thermostats were calibrated at 45° to 60°C to cool about 0.2° to 0.5°C in 24 hours. Small heating effects due to calibration were therefore likely to occur with seeds at the beginning of a heating trial at room temperature, but these were manifested by linear or slightly hyperbolic temperature increases. In contrast, temperature-time curves yielded by spontaneous heating phenomena were always *exponential* in form. Accordingly, heating effects obtained from room temperature to that at which the thermostat was calibrated (45° to 60°C) would be a composite of a slight and virtually linear heating effect due to the calibration anomaly, and a large and exponential form of heating due to biological agencies. Furthermore, heating effects in temperature ranges at and above the calibration temperature (45° to 60°C) are not only entirely due to spontaneous thermogenic processes in the seeds, but are at the same time underestimations of the true maximum heating potential, inasmuch as the heat loss from the system increases as the temperature exceeds the calibration temperature.

Materials and Methods

Materials. The soybeans used in this study included: (1) a sound sample of Illini soybeans of the 1942 crop, testing 94% germination, grown in Illinois, and (2) an exceptionally sound, well-matured sample of Wisconsin Manchu soybeans grown for seed purposes at University Farm, St. Paul, Minnesota, in 1943, and testing 93 to 95% germination.

METHODS:

Conditioning Soybean Samples. The seed samples were conditioned to the required moisture levels by the direct addition of water;

moisture values were determined by means of a two-stage method as described in a previous communication (Milner and Geddes, 1945).

Examination of Soybeans for Fungi. Estimation of the number of seeds in a given sample infected with molds, as well as identification of the prevalent mold types, was carried out by the surface-sterilization and plating technique described by Milner, Warshowsky, Tervet, and Geddes (1943).

Chemical Analyses. Analyses for crude protein, crude ether extract, and for total, reducing, and nonreducing sugars were carried out on finely ground soybean samples by the methods outlined for wheat flour in *Cereal Laboratory Methods* (4th ed., 1941). Nonprotein nitrogen was measured by the trichloracetic acid method described by Becker, Milner, and Nagel (1940).

Spontaneous Heating and Respiration of Illini Soybeans

A study of the spontaneous heating and the respiratory phenomena exhibited by Illini soybeans was carried out with a sample weight of 550 g of seed at an initial moisture value of 22.8% (equilibrium relative humidity approximately 92%). A number of 100-g portions of the same bulk sample in small bottles were placed in the air bath and independently aerated for the purpose of sampling the seeds at various stages in the heating trial.

The data for this trial are given graphically in Figure 2. In this and subsequent figures, the scale for daily rates of carbon dioxide production is shown on the left margin while that for the daily recorded temperature is shown on the right. An additional graph for the respiratory quotients is shown at the top of each figure. Chemical and germination values, and the degree of microfloral infection, at various intervals in the trial, are shown in Table I.

The initial aeration rate of 2,000 ml per day proved to be insufficient, as respiratory inhibition was apparent from the slope of the respiration curve during the first few days. On the seventh day when the interseed air carbon dioxide and oxygen values had reached 18.8% and 2.2%, respectively, the aeration rate was doubled by increasing the speed of the aeration device, and samples of the respired air were accordingly taken twice daily until the end of the experiment.

The moisture content of the seeds in the Dewar flask at the end of the trial was 22.1% (initial value 22.8%). The initial and final weights of the sample at these moisture values were 550 and 542.5 g, respectively, representing dry matter weights of 428.4 and 424.6 g. The apparent dry matter loss during the trial was therefore 3.8 g.

A calibration check of the adiabatic thermostat at the end of the trial showed it to be cooling at the rate of 0.3°C per 24 hours from an initial water temperature of 55.8°C.

The data show clearly that the parallel increases in temperature and carbon dioxide production are associated with an increasing mold population until a temperature of 50°C is reached. At this point, thermal killing of the biological respiratory agencies sets in with a corresponding rapid decrease in respiration. During this initial heat-

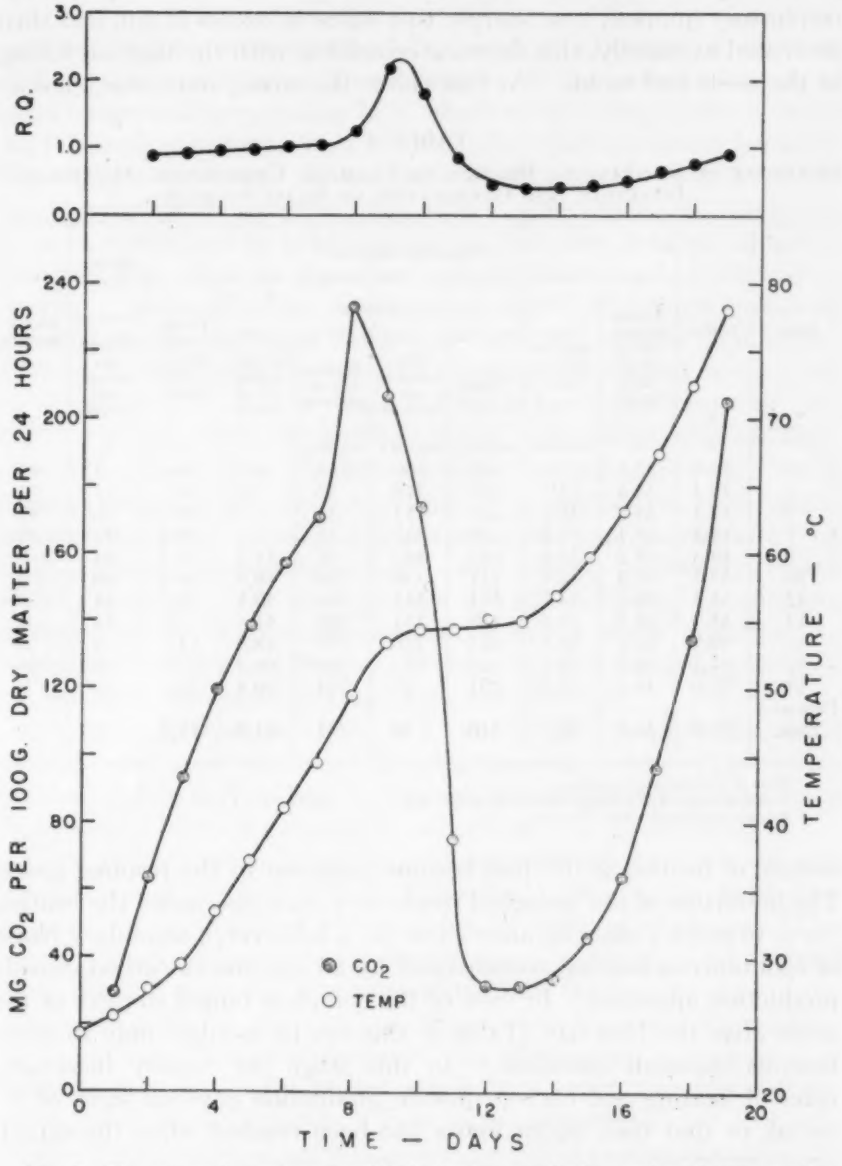


Fig. 2. The course of spontaneous adiabatic heating, respiration, and respiratory quotient exhibited by Illini soybeans, under adiabatic conditions, at 22.8% moisture. The aeration was doubled on the seventh day.

ing phase the respiratory quotient approaches a value of 1.0 which in this case was attained immediately prior to thermal killing. This regular increase in the respiratory quotient is probably indicative of the increasing contribution of the molds to the total respiration, as pointed out in previous studies at constant temperatures by Milner and Geddes (1945). As the temperature increased beyond this point, the respiratory quotient rose sharply to a value in excess of 2.0, and then decreased as rapidly, this decrease coinciding with the thermal killing of the seeds and molds. At this point, the strong sour odor, charac-

TABLE I

INFLUENCE OF SPONTANEOUS HEATING ON CHEMICAL COMPOSITION, MICROFLORAL INFECTION, AND GERMINATION OF ILLINI SOYBEANS

Days	Temp.	Chemical analyses							Microflora	Germination
		Crude protein (N × 6.25 dry basis)	Non-protein nitrogen	Sugars				Crude ether extract (dry basis)	Seeds infected with fungi[2]	
				Total[1]	Non-reducing as sucrose	Reducing as glucose	Reducing as % of total			
	°C	%	mg/10 g	mg/10 g	mg/10 g	mg/10 g	%	%	%	%
0	24.4	35.4	22.4	444	400	44	10.0	21.6	3	93
3	29.4	35.4	21.2	410	378	32	7.8	19.5	11	73
5	37.1	35.8	21.2	405	373	32	6.9	20.2	16	52
7	44.4	35,1	22.2	378	347	31	8.1	20.2	27	30
8	49.3	35.2	23.6	433	382	51	11.8	20.3	64	0
10	53.7	35.4	28.2	417	334	83	19.9	19.4	40	0
12	55.1	35.7	34.7	491	245	246	50.1	15.3	14	0
13	55.2	35.8	32.4	438	251	187	42.6	14.9	13	0
15	59.7	36.1	32.3	443	226	217	48.9	13.6	9	0
17	67.3	36.3	35.7	432	172	260	60.3	12.0	0	0
19	77.0	36.6	35.0	291	27	264	90.8	10.5	0	0
Dewar[3] flask	77.0	36.6	37.7	310	59	251	81.0	11.2	0	0

[1] Sum of sucrose and glucose.
[2] The majority of the fungi were *Aspergillus* spp.
[3] Sample analyzed after 19 days.

teristic of heating grain, first became apparent in the respired gases. The inhibition of the biological respiratory agencies caused the heating curve to reach a plateau; after a few days, however, a secondary phase of spontaneous heating accompanied by an increase in carbon dioxide production appeared. In view of the complete fungal sterility of the seeds after the 15th day (Table I) this can be ascribed only to spontaneous chemical oxidation.[4] In this stage the rapidly increasing rates of heating and carbon dioxide production gave no signs of reversal, or that their upper limits had been reached when the experi-

[4] Subsequent experiments showed that the bacteria indigenous to these soybeans would not proliferate at this humidity level.

ment was terminated on the 19th day. In this last stage of spontaneous chemical heating, the respiratory quotient, which had fallen to extremely low values (0.4 to 0.5) following thermal inactivation of respiration, rose slowly as the chemical heating increased. It is noteworthy that this range of 0.4 to 0.5 in the respiratory quotient agrees precisely with the values obtained by Milner and Geddes (1945) in respiratory trials with soybeans at 50°C.

These results serve to elucidate the cause of failure of previous studies on microbiological thermogenesis and spontaneous heating to yield temperatures exceeding 55°C where molds or mesophilic bacteria are the cause of heating, or above 70°C where thermopiles are involved. The plateau reached by the heating curve due to the cessation of biological respiration indicates that rather strict adiabatic conditions must be maintained in order that the spontaneous chemical oxidation, which is very weak at these low temperatures, may be allowed to develop. Sallans, Sinclair, and Larmour (1944) carried out studies on the spontaneous heating of flaxseed and sunflower seed in an adiabatically controlled thermostat based on the design of Working as described by Ramstad and Geddes (1942). A maximum temperature of only 52°C, a value within the mold thermal death range, was attained. It would appear that adequate aeration was not provided. These workers point out that under conditions of oxygen starvation, a regular relationship between heating and respiration rate as measured by carbon dioxide production is not to be expected. However, in the present studies where aeration was usually sufficient to maintain interseed carbon dioxide concentrations below levels inhibitory to respiration (Milner and Geddes, 1945), a direct relationship between temperature and respiratory increases was observed.

Carlyle and Norman (1941) also used an adiabatically controlled thermostat in their studies on microbial thermogenesis. These workers were unable to show heating beyond 70°C in experiments involving thermophilic bacteria growing on hay at 100% relative humidity.

The present study seems to add confirmation to the data and theories of Haldane and Makgill (1934) and Hoffman (1935, 1940) concerning the phenomenon of spontaneous chemical oxidation. The former writers held moist hay at temperatures just beyond the thermal death point of microorganisms and found that the gas exchange of the chemically oxidizing material was characterized by a very low respiratory quotient. Hoffman confirmed this observation, but showed in addition that previously heated sterile hay would give increasingly higher respiratory quotients if held at successively higher temperatures. Both of these observations are capable of explanation from the data obtained in the present trial with soybeans, in which the heating

beyond the microfloral death range was entirely spontaneous. At temperatures slightly above that required to kill molds (50°–55°C), the respiratory quotients were very low (0.4 to 0.5), indicative of strong oxygen absorption and low carbon dioxide evolution. Higher temperatures apparently cause a breakdown of the previously formed, highly oxidized materials to yield a strong evolution of carbon dioxide. The oxygen requirement for complete oxidation in this stage would be correspondingly low, with the result that the respiratory quotient would rise.

The germination data, chemical analyses, and degree of mold infection (Table I) obtained on samples removed at various stages during the trial lend support to the foregoing interpretation of the heating and respiratory changes. The slight increase noted in the protein content is probably a reflection of a decrease in sample dry matter weight due to selective respiratory utilization of carbohydrates. Total sugars show a slow decrease in the initial biological heating stage to 49.3°C followed by a sharp rise in the initial phase of spontaneous chemical heating. This increase may only be an apparent one since it might well be due to thermal cleavage of large carbohydrate molecules such as sucrose, stachyose, raffinose, and pentosans, to yield numerous small reducing fragments. Such reducing fragments would absorb oxygen strongly which might account in large measure for the low respiratory quotient of the gas exchange in this phase. The succeeding heating period then brings about a rapid decline in total sugars. Nonreducing substances calculated as sucrose show a slow decline in the biological stage of heating and a marked decline as chemical heating sets in. Reducing substances similarly decline slightly in the biological heating phase but increase drastically at the expense of the nonreducing carbohydrates in the region of spontaneous chemical heating. The reducing sugar values in this phase may be an index of the thermal oxidative cleavage already discussed.

The crude fat fraction showed virtually no change until the temperature-time curve reached a plateau signifying the end of biological respiration. From this point the extractable oil fell sharply from 20.3% to 10.5% at the end of the trial when the temperature had reached 77°C. If it were assumed that this loss of oil were due to respiration, a disappearance of $\frac{(20.3 - 10.5)}{100} \times 428.4$ or 42.0 g of dry matter would be expected. As indicated previously, the actual loss of dry matter was only 3.8 g. Assuming a respiratory quotient for carbohydrates of 1.0, the total carbon dioxide evolution over the course of the trial would require a loss of 6.1 g in dry weight. It must be concluded that not only was the oil fraction *not* materially dimin-

ished, but that the loss of carbohydrate was less than would be expected on the basis of carbon dioxide evolution. It seems possible that the low respiratory quotient of the gas exchange which occurred following biological inhibition may in part be associated with an absorption of oxygen by the unsaturated soybean oils, to yield oxidation polymers. Such polymers would be only slightly soluble in petroleum ether.

This experiment indicates that the initial stage of heating in damp seeds may be largely attributed to mold activity. The temperature at which biological respiration was inactivated corresponds closely to that found by Carlyle and Norman (1941) for several mold species which they designated as mesophils. The study has experimentally demonstrated that spontaneous heating occurs in biological materials, following the thermal killing of the microflora initially present. Browne (1933) has pointed out that this heating due to spontaneous chemical oxidation, if allowed to continue without heat dissipation, results in spontaneous ignition at about 280°C.

Replicability of Spontaneous Heating Studies

The heating and respiratory phenomena exhibited by duplicate samples of damp soybeans were studied on completion of a second adiabatic apparatus which was put into operation following the first heating experiment. For this study, two one-pint Dewar flasks and Illini seed samples of 250 g were used, in order to assure adequate aeration at the 2,000 ml daily rate through the entire course of the trial. The soybean sample was conditioned to 18.4% moisture (85% relative humidity) over only a 24-hour period as contrasted to the three-day conditioning period used in the previous trial.

The data shown graphically in Figure 3 followed closely the pattern of respiratory and heating phenomena shown in the first trial, and demonstrated that excellent replication is possible. This experiment revealed that when soybean seeds are placed into respirometer bottles rather promptly after conditioning, a respiration lag period is shown. In addition, the phenomenon of high initial respiratory quotient, which Milner and Geddes (1945) found was associated with the release of carbon dioxide from freshly wetted soybeans, appears.

Influence of Heavy Inocula of Microflora on the Heating and Respiration of Normal Soybeans

To study the influence of heavy inocula of microflora on the spontaneous heating of normal seeds showing low microfloral contamination, a 250-g portion of the high quality Wisconsin Manchu seed was conditioned with sterile water to 18.0% moisture (84% relative humidity)

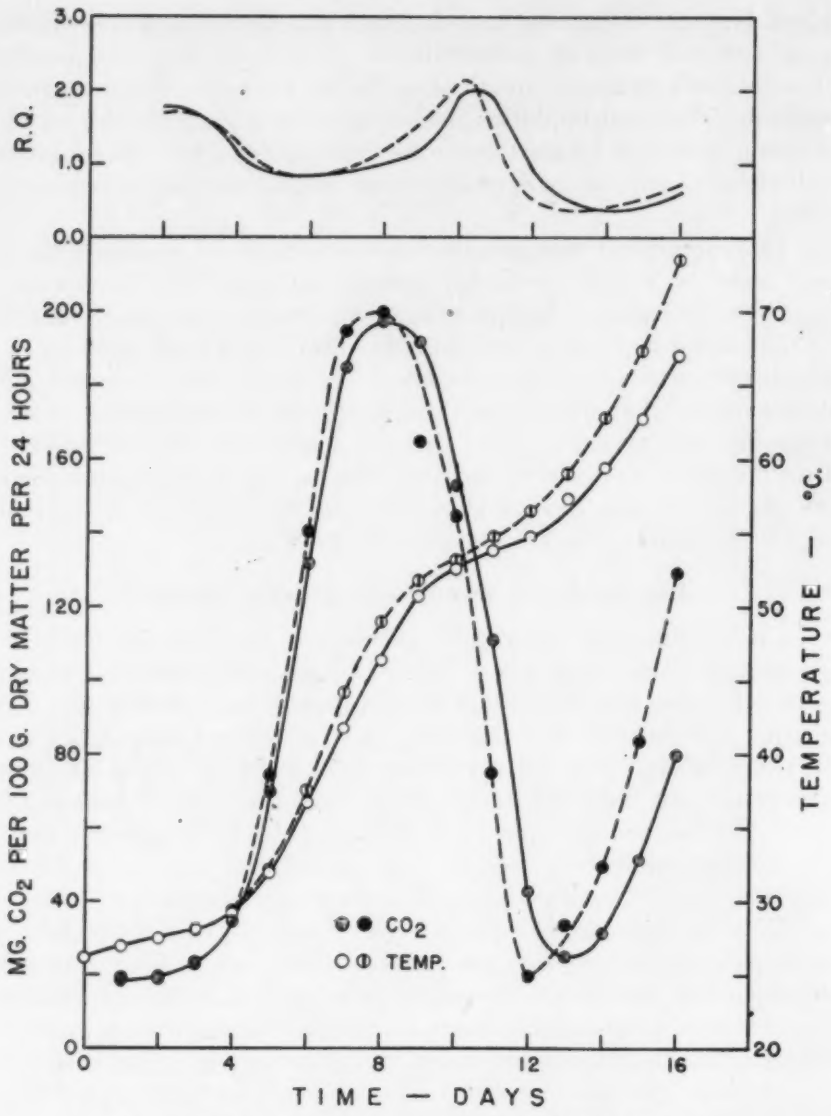


Fig. 3. The replicability of spontaneous, adiabatic heating studies with Illini soybeans at 18.4% moisture.

while a similar subsample received the same volume of an aqueous suspension of microflora representative of the variety of species present in the normal seed, which had been previously isolated from soybeans and propagated in pure culture. The inoculum included spores of *Aspergillus flavus*, *A. ochraceus*, *A. glaucus*, *A. fumigatus*, a *Penicillium* spp., a brown Aspergillus isolated from soybean meal, as well as broth cultures of unidentified bacteria from discolored soy-

bean cotyledons, *Bacillus mesentericus*, and unidentified rod-shaped bacteria obtained from soybeans used in the previous study. Smaller portions of both seed lots in small bottles were placed in the respective air baths, and independently aerated as in the first trial.

As indicated in Figure 4, both the inoculated and noninoculated seed showed very slow initial heating; significant increases in tempera-

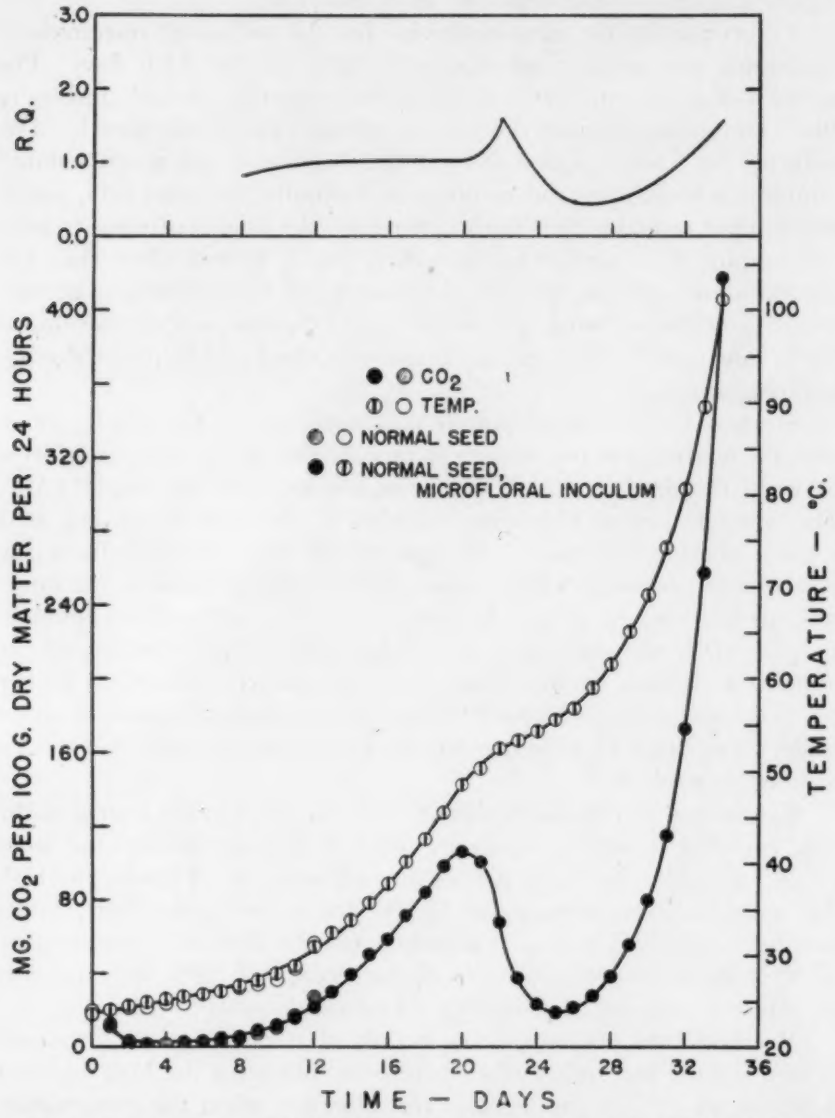


Fig. 4. The spontaneous adiabatic heating and respiratory characteristics of Wisconsin Manchu soybeans at 18.0% moisture and of the same seed heavily inoculated with mixed microflora. The trial for the noninoculated seed was discontinued on the 12th day. The curve for the respiratory quotients applies to the inoculated sample.

ture and respiration occurred only after the eighth day. From this time to the 12th day the course of the heating and respiration was virtually identical for both. The probable reason for this common trend became apparent when on the 11th day, subsamples from both lots were removed and the seeds were cultured in sterile petri dishes. Both the inoculated and noninoculated seed gave rise to a grey-green Aspergillus tentatively identified as *A. glaucus*.

Unfortunately the control device for the adiabatic respirometer containing the noninoculated sample failed on the 13th day. The samples of seed removed from both respirometers at this time were 100% contaminated with the grey-green mold previously noted. The data for the 13-day period showed the inoculated and noninoculated samples to be heating and respiring at virtually the same rate, and it seems quite probable that both samples would have continued to heat and respire in a similar manner thereafter. It was clear that the noninoculated sample, which in a dry state before conditioning showed only 5% of the seeds to be infected by *A. glaucus*, quickly developed 100% infection by this fungus when conditioned and held in the adiabatic apparatus.

Study of the inoculated sample was continued for 34 days, by which time the heating was progressing so rapidly that in the 24-hour interval between the 33rd and 34th days the temperature increased 11.5°C. The temperature at this time was 101°C and was increasing at a rapidly accelerating pace. Marked distillation of water from the system was underway while carbon dioxide concentration in the interseed air had risen to 21%. Increased aeration could not be provided. In view of these factors and the nonfire resistant construction of the apparatus, it was deemed hazardous to carry the study to higher temperatures. There seems little question that if adequate aeration could have been provided, ignition temperatures would have been reached in a few days.

Continued microfloral examination of the seeds in the course of the trial revealed that the grey-green mold *A. glaucus* proliferated more readily than all other mold species in the inoculum. Thus by the 17th day when the temperature was 40.1°C, the seeds showed 100% infection by molds, 95% being *A. glaucus*. On the 19th day (temperature 45.6°C) the only mold arising from the seeds, still 100% infected, was *A. glaucus*. On the 21st day (51°C) no viable molds were found.

Bacterial infection, consisting mainly of *B. mesentericus*, decreased in terms of the percentage of seeds infected following the 17th day, but persisted on 15% of the seeds to the 32nd day when the temperature was 80°C. At the end of the trial (101°C) all the seeds were sterile. It must be stressed that no estimate was made of the *number* of

bacterial cells. However, no visual evidence of bacterial proliferation on the samples was noted at any time, in contrast with the heavy mycelial growth shown by the molds prior to thermal killing. In a subsequent experiment, six unidentified bacterial species, which were isolated from the interior tissues of soybeans and grown in pure culture, were used to inoculate autoclave-sterilized soybeans containing 21% moisture (90% relative humidity at 25°C). No heating or respiration attributable to bacterial growth at this humidity level was noted.

Certain points of difference in the respiration and heating characteristics of the Illini and Wisconsin Manchu soybean seeds are to be noted. The Wisconsin Manchu seed showed a very long lag period both for heating and carbon dioxide production as compared to the Illini sample used in the previous trial. Similarly, the Manchu seed showed a considerably lower maximum biological carbon dioxide production rate (106 mg as compared to 200 mg) at approximately the same moisture value. It might be inferred that this particular sample of high quality Wisconsin Manchu soybeans provided a less hospitable medium for the molds than did the Illini sample.

Influence of Surface Sterilization and Mold Inoculation on the Spontaneous Heating and Respiration of Soybeans

The results of the previous trial showed that even the slight microfloral contamination of untreated normal soybean seeds yielded as rapid mold proliferation, respiration, and heating, as did heavy inocula of microflora. In an attempt to inhibit the growth of the normal seed microflora in order to study seed respiration and heating in the absence of that due to the molds, a lot of the Wisconsin Manchu soybeans was sterilized by shaking in a solution of mercuric chloride (1–1,000) for one-half minute. After decantation the seeds were washed for one minute with sterile water and allowed to drain. After 14 hours in a sealed jar, the moisture value of the seed was 19.7% (equilibrium relative humidity 87%). A 250-g sample was transferred to a sterile Dewar flask and smaller portions were placed into sterile 8-ounce bottles for insertion into the adiabatic respirometers for a respiration and heating trial. Another portion of the same sterilized, conditioned seed was heavily inoculated with spores of a pure culture of *A. flavus* and the respiratory and heating characteristics studied in the other adiabatic respirometer.

The respiration and heating data are presented in Figure 5. The surface-sterilized and surface-sterilized reinoculated seeds heated and respired in a virtually identical manner. As the trial proceeded, both seed lots proved to be contaminated with *A. flavus* and *A. glaucus*, the percentage of seeds infected being similar for both at similar time

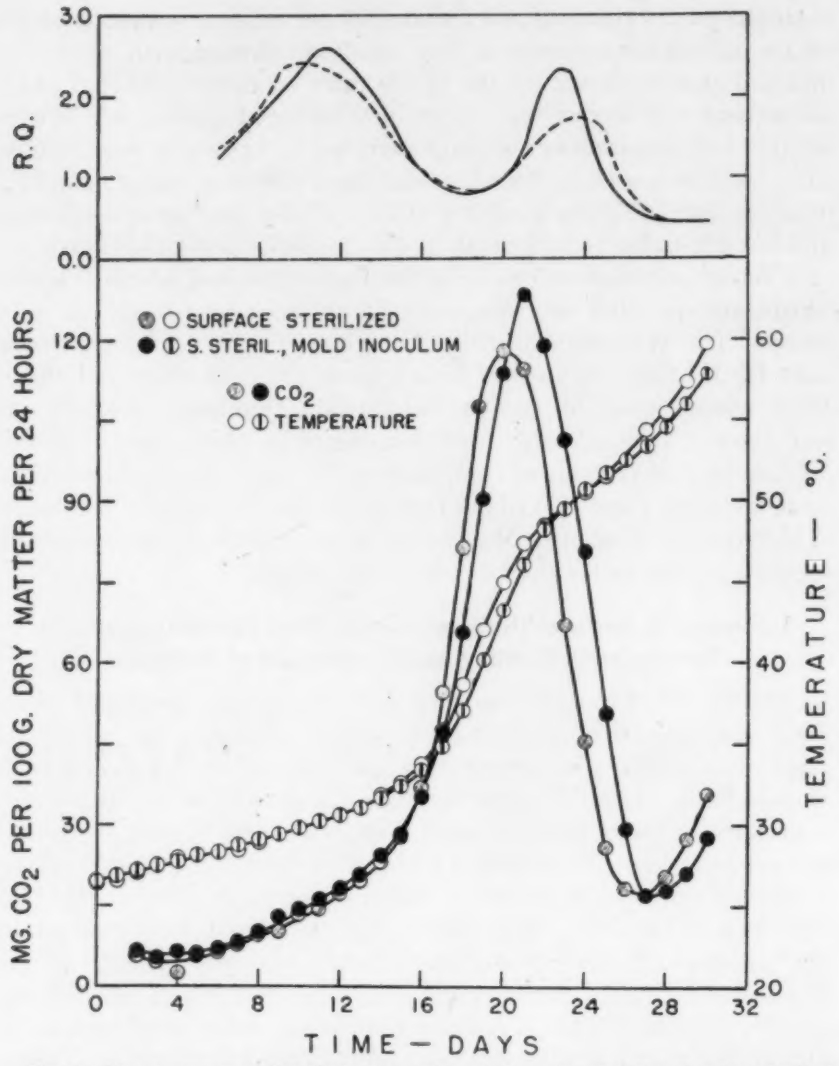


Fig. 5. The spontaneous adiabatic heating and respiratory characteristics of surface-sterilized Wisconsin Manchu soybeans at 19.7% moisture, and of the same seed inoculated with *Aspergillus flavus*.

periods. It was apparent that surface sterilization by the technique used was ineffective against mold contaminants, which apparently existed well within the seed coats and were unaffected by sterilization at the surface. This result is in complete agreement with the observation of Mead (1941) who found that seeds treated with mercurial dusts or formalin to kill surface microfloral contaminants would subsequently yield growths of mold, arising from the more deep-seated infections, when cultured on moist media.

Spontaneous Heating and Respiration of Autoclave-Sterilized Soybeans with and without Mold Inoculation

The failure of the surface sterilization technique to provide a means of studying independently the respiration due to molds and seeds suggested a trial involving the inoculation of sterile seeds with molds to obtain data on the respiration and thermogenic behavior of the microorganism itself. It was also of interest to investigate the minimal temperatures at which the secondary spontaneous chemical heating phase would occur.

An adiabatic heating and respiration trial was conducted on a conditioned lot of the Wisconsin Manchu seed which had been autoclaved for one hour at 120°C (together with the requisite Dewar flasks, glass wool-plugged connections, 8-ounce bottles, and other accessories) and then inoculated with spores of a pure culture of *A. flavus*. A subsample from the same lot of autoclaved seeds, but uninoculated, was used in the other adiabatic apparatus. The moisture content of this grain was 20.4% (89% relative humidity).

Samples of both lots of seeds removed from the sterile 8-ounce bottles on the seventh day of trial showed the inoculated seeds to be 100% contaminated with *A. flavus*, while the sterilized noninoculated seeds were completely sterile.

The respiration and heating data for this trial are given in Figure 6. The course of the carbon dioxide evolution, due entirely to the metabolism of the mold *A. flavus*, is almost identical to that given by normal seed where the organism was a natural contaminant. The heating curve shows similar characteristics to that of normal seeds, including the attainment of a plateau resulting from the thermal death of the microflora, which is followed by secondary heating due to chemical oxidation. The curve for the respiratory quotients is somewhat different in that the sharp peak yielded by normal seeds following biological thermal killing is absent. This phenomenon seems to be associated with temperature-induced evolution of carbon dioxide from initially normal seeds, and is therefore not related to the respiratory exchange or the thermal death of the mold population.

The apparent slight heating shown by the sterile seeds in the first 13 days of the trial is ascribed to the calibration anomaly previously discussed. No detectable carbon dioxide evolution appeared in this temperature range (22° to 25°C). To ascertain if chemical heating was possible without initial biological activity, the auxiliary heaters of the thermostat containing the sterile seeds were turned on for a short period, thus raising the temperature of the seeds in the Dewar flask to 31.1°C; the thermocouple control was then reestablished.

Subsequently, similar temperature increases were applied, as shown in Figure 6.

The slight temperature increase beginning at 31.1°C (13th to 21st day) may be accounted for by the calibration anomaly; however, detectable amounts of carbon dioxide in the order of 0.5 mg per 100 g dry matter weight appeared. At 40.5°C (22nd day) no temperature change was recorded over a two-day interval, but the carbon dioxide

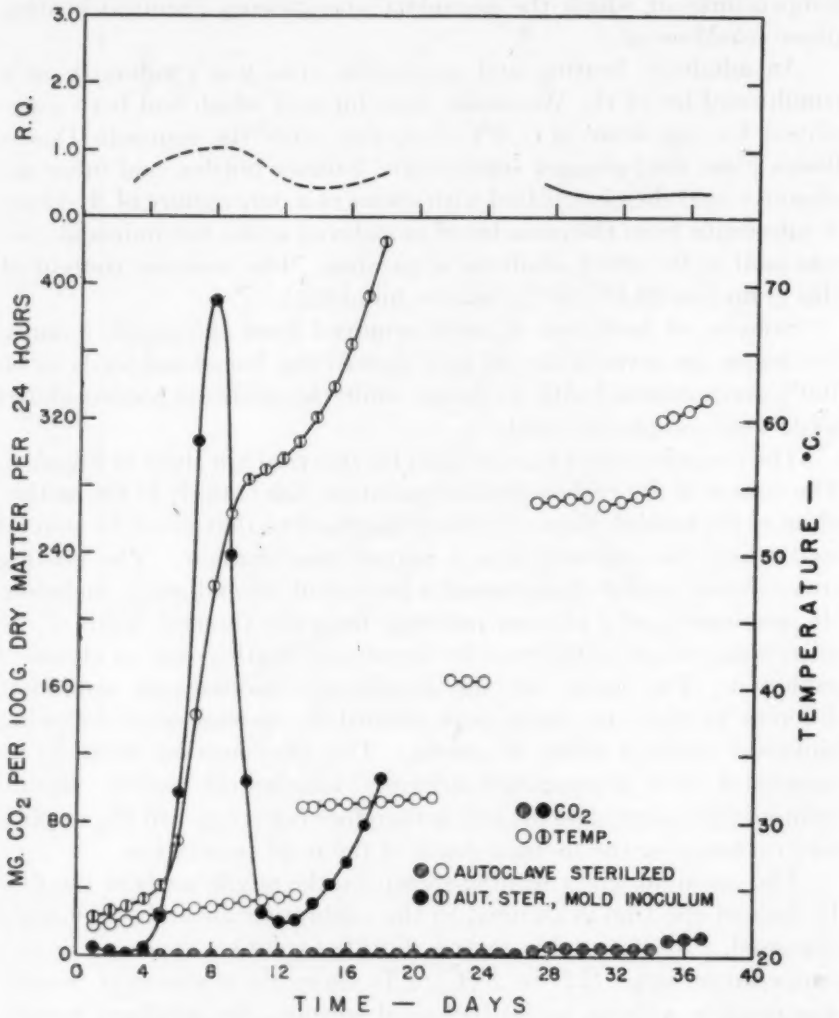


Fig. 6. The spontaneous adiabatic heating and respiratory characteristics of autoclave-sterilized Wisconsin Manchu soybeans at 20.4% moisture, and of the same seed reinoculated with *Aspergillus flavus*. In the latter case, the sample was artificially adjusted to different temperatures after various time intervals and the spontaneous heating at each temperature level determined for several days. The R.Q. curve shown as a dotted line pertains to the mold-inoculated sample; the curve for the sterile sample is shown as a solid line.

production rose to 1.2 mg. The constant temperature during this interval indicated this temperature to be close to the value at which virtually adiabatic calibration conditions existed, and that a balance existed between heat production and loss.

At 53.8°C (27th day) definite spontaneous heating was noted, the carbon dioxide production being 4.7 mg. Even stronger spontaneous heating appeared at 60°C, a temperature where cooling would occur on calibration. The mean carbon dioxide production for the three days following establishment of this temperature was 10.2 mg. The respiratory quotient of this gas exchange was 0.4, corresponding to that shown by normal or autoclaved inoculated seeds in the initial ranges of spontaneous chemical heating, following the thermal inactivation of biological agencies. This experiment demonstrates that carbon dioxide production due solely to chemical oxidation occurs in the temperature range where microorganisms are active, and that spontaneous heating due to chemical oxidation is possible at higher temperatures even without the normal preliminary activity of the microorganisms.

Discussion

Two distinct ranges of temperature increase have been shown to occur with dormant soybeans heating spontaneously under adiabatic conditions at humidity levels sufficiently high as to favor the proliferation of mold fungi, yet too low to cause bacterial growth. The first heating phase, which is a consequence of the respiration and metabolism of the proliferating molds, ends when the thermal death range (50°–55°C) of these microflora is reached. The species *Aspergillus glaucus* and *A. flavus* were primarily involved. The second heating stage, concealed in its lower range under natural conditions by the upper temperature range achieved by the molds, appears clearly at temperatures higher than the thermal death range of the molds and seeds. The fact that autoclave-sterilized seeds would heat and produce carbon dioxide spontaneously, in a manner directly similar to normal seeds where preliminary microfloral activity occurs, indicates that this secondary heating cannot be ascribed to enzymic or other biological phenomena. Under adiabatic conditions and with sufficient aeration, this secondary spontaneous heating due to nonbiological oxidation apparently has no upper limit short of the ignition temperature of the seed materials.

The present studies have shown that a close parallelism between rate of heating in terms of temperature increment and carbon dioxide production exists in all stages when adequate aeration is provided. This is evident from a comparison of the daily temperature increments with the respiratory rates.

The inadequacy of surface sterilization techniques in preventing growth of the contaminating mold spores precluded study of seed respiration itself. On the other hand, heating studies conducted by inoculating autoclave-sterilized soybeans with spores of a pure culture of *Aspergillus flavus* showed that the molds are primarily responsible for the characteristic course of spontaneous heating and respiration noted with normal soybeans. Except for certain minor respiratory phenomena, such as a brief initial evolution of carbon dioxide due to wetting of the seed, and a similar dissipation of this gas immediately following the thermal death point of the seeds as evidenced by a sharp peak in the respiratory quotient, the respiration of the dormant seed seems to play virtually no part in the heating. Under practical conditions, other than those where the moisture content would be sufficiently high to cause seed germination, the respiration of the seed itself is insignificant in storage and heating phenomena.

Of the changes in the various chemical factors in the seeds which were assayed at intervals in the course of the heating trials, the most notable was the decrease in the petroleum ether soluble fraction which occurred in the temperature range of spontaneous chemical heating. The data indicate that this loss of oil is only an apparent one since no consequent loss of dry matter weight takes place. This effect is probably due to oxidative and thermal polymerization of the unsaturated seed oils, rendering them less soluble in petroleum ether. This phenomenon may be responsible for the ease with which spontaneous chemical heating occurs in soybeans, following thermal inactivation of the biological thermogenic agencies.

It is speculated that the apparent increase in total reducing substances with the onset of chemical heating may be due to thermal cleavage of carbohydrate molecules to yield numerous smaller reducing fragments of high oxygen-absorbing capacity. Such carbohydrate degradation products might be classed with the pyrophoric compounds whose formation as a preliminary to spontaneous combustion has been postulated by Miehe (1911) and Browne (1929).

Summary

Spontaneous heating and associated respiratory characteristics of soybeans at humidity levels favorable to mold growth but unfavorable to bacterial proliferation were studied in an apparatus which maintained continuously controlled adiabatic and aeration conditions, over time intervals up to 37 days. An initial temperature increase to 50°–55°C and parallel respiratory increase were directly associated with the proliferation of the molds *Aspergillus glaucus* and *A. flavus*.

A secondary spontaneous heating phase due to nonbiological oxida-

tion was demonstrated, which appeared to have no limit short of ignition temperatures, and was closely paralleled by the extent of carbon dioxide evolution.

Surface sterilization of the seeds failed to eliminate mold infection, whereas inoculation of autoclave-sterilized soybeans with spores of *Aspergillus flavus* yielded heating and respiration curves virtually identical to those of normal seeds.

Spontaneous heating of sterile seeds in which no microfloral activity had occurred was demonstrated. Chemical changes in the heating seeds assayed at intervals in the course of the trials indicated a disappearance of total sugars in the initial biological phase of heating and an increase in reducing substances in the initial spontaneous chemical heating phase. The petroleum ether-soluble fraction remained virtually unchanged in the biological heating stage but decreased markedly in the chemical heating phase, without a corresponding loss in dry matter content of the seeds.

Respiratory quotients associated with the gas exchange during the spontaneous chemical heating phase suggest the occurrence of thermally induced oxidative cleavage of carbohydrates as well as oxidative polymerization of the seed oils.

Acknowledgment

The authors are indebted to Mr. E. A. Andrews and Dr. C. M. Christensen, of the Division of Plant Pathology, University of Minnesota, who provided the mycological data.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods (Fourth Ed.). American Association of Cereal Chemists, Lincoln, Neb.
Bailey, C. H.
1940 Respiration of cereal grains and flaxseed. Plant Physiol. **15**: 257–274.
——, and Gurjar, A. M.
1918 Respiration of stored wheat. J. Agr. Research **12**: 685–713.
Bakke, A. L., and Henson, E. R.
1933 Relation of fungi to respiration and fermentation occurring in stored hay. Iowa Agr. Expt. Sta. Report on Agr. Research, p. 51.
——, and Noecker, N. L.
1933 The relation of moisture to respiration and heating in stored oats. Iowa Agr. Expt. Sta. Research Bull. **165**: 319–336.
Becker, H. C., Milner, R. T., and Nagel, R. H.
1940 A method for the determination of non-protein nitrogen in soybean meal. Cereal Chem. **17**: 447–457.
Browne, C. A.
1929 The spontaneous combustion of hay. U.S.D.A. Tech. Bull. 141.
1933 The spontaneous heating and ignition of hay and other agricultural products. Science **77**: 223–229.
Carlyle, R. E.
1940 Measurement of the heat liberated in the biological decomposition of plant materials. Iowa State Coll. J. Sci. **15**: 60–62.
——, and Norman, A. G.
1941 Microbial thermogenesis in the decomposition of plant materials. Part II. Factors involved. J. Bact. **41**: 699–724.

Cohn, F.
1890 Ueber Warme Erzeugung durch Schimmelpilze und Bakterien. Jahresber. Schles. Gesell. (Breslau) **68**: 23–29. Cited by Browne (1929). (Original not seen.)
Darsie, M. L., Elliot, C., and Peirce, G. J.
1914 A study of the germinating power of seeds. Bot. Gaz. **58**: 101–136.
Gilman, J. C., and Barron, D. H.
1930 Effect of molds on temperature of stored grain. Plant Physiol. **5**: 565–573.
Haldane, J. S., and Makgill, R. H.
1934 The spontaneous oxidation of coal and other organic substances. J. Soc. Chem. Ind. **53**: 359–367 T.
Harrison, J. W.
1934 Thermogenesis in hay inhabiting fungi. Iowa State Coll. J. Science **9**: 37–60.
Hoffman, E. J.
1935 Oxidation and gas formation in the spontaneous heating of hay. J. Agr. Research **51**: 527–546.
1940 Thermal decomposition of undercured alfalfa hay in its relation to spontaneous ignition. J. Agr. Research **61**: 241–257.
James, L. H.
1927 Studies in microbial thermogenesis. I. Apparatus. Science **65**: 504–506.
——, Rettger, L. F., and Thom, C.
1928 Microbial thermogenesis. II. Heat production in moist organic materials with special reference to the part played by microorganisms. J. Bact. **15**: 117–141.
Mead, H. W.
1941 The disinfecting value of fungicides used for treating cereal seeds and their influence on growth. Sci. Agr. **21**: 717–726.
Miehe, H.
1911 Ueber die selbsterhitzung des Heues. Arb. deut. landw. Gesell. **196**: 1–47. Cited by Hoffman (1935). (Original not seen.)
Milner, M., Warshowsky, B., Tervet, I. W., and Geddes, W. F.
1943 The viability, chemical composition and internal microflora of frost damaged soybeans. Oil and Soap **20**: 265–268.
——, and Geddes, W. F.
1945 Grain storage studies. II. The effect of aeration, temperature and time on the respiration of soybeans containing excessive moisture. Cereal Chem. **22**: 484–501.
1946 Grain storage studies. III. The relation between moisture content, mold growth, and respiration of soybeans. Cereal Chem. **23**: 225–247.
Norman, A. G., Richards, L. A., and Carlyle, R. E.
1941 Microbial thermogenesis in the decomposition of plant materials. Part I. An adiabatic fermentation apparatus. J. Bact. **41**: 689–697.
Ramstad, P. E., and Geddes, W. F.
1942 The respiration and storage behavior of soybeans. Minn. Agr. Expt. Sta. Tech. Bull. 156.
Sallans, H. R., Sinclair, G. D., and Larmour, R. K.
1944 The spontaneous heating of flaxseed and sunflower seed stored under adiabatic conditions. Can. J. Research **F22**: 181–190.
Wedberg, S. E., and Rettger, L. F.
1941 Factors influencing microbial thermogenesis. J. Bact. **41**: 725–743.

THE EFFECT OF PRESSURE ON MACARONI DISCS: SIZE AND NUMBER OF AIR BUBBLES IN RELATION TO LIGHT TRANSMISSION [1]

GLENN S. SMITH,[2] R. H. HARRIS,[3] ETHEL JESPERSON,[4] and L. D. SIBBITT [5]

(Received for publication April 17, 1946)

All macaroni products normally contain air bubbles. Air is introduced in the process of mixing the doughs, but bubbles cannot be found on the surface of macaroni products, perhaps because of surface tension. Microscopic examination of the fractured surfaces, however, reveals numerous bubbles, 200 to 400 μ in diameter. This paper presents evidence that the size and number of these air bubbles are related to the translucency of the finished macaroni.

Translucency is very important in macaroni products. The endosperm of a vitreous wheat kernel is translucent (Alsberg, 1939), and even individual starch grains under the microscope are translucent. However, the appearance of translucency to the naked eye is lost in processing the semolina and not recovered until pressure is applied to the dough.

The significance of pressure in developing translucency was pointed out by Fifield, Smith, and Hayes (1937) in describing the macaroni disc method, a technique proposed for studying the quality of small samples of durum. Cunningham and Anderson (1943a) studied the effects of processing conditions on paste properties of macaroni discs as measured by the optical coefficient of opacity. They concluded that pressing, mixing, and absorption had the greatest influence on opacity (or translucency). The latter workers used bubble size in determining the pressure needed to yield an internal structure comparable with that of commercial macaroni products. They suggested that longer mixing time increases opacity chiefly because it increases the number of minute air bubbles incorporated into the paste.

Before pressing, macaroni dough is dull in color, resembling putty, and the first few inches of the extruded product from the die are still white in color and opaque. Gradually, however, as more material is extruded this color becomes clear and translucent. Microscopic examination of the internal structure of this macaroni shows that the

[1] Joint contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Cereal Technology, North Dakota Agricultural Experiment Station.

[2] Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, field headquarters at Fargo, North Dakota.

[3] Cereal Technologist, North Dakota Agricultural Experiment Station.

[4] Research Assistant, Department of Cereal Technology, North Dakota Agricultural Experiment Station.

[5] Assistant Cereal Technologist, North Dakota Agricultural Experiment Station.

opaque section is honeycombed with numerous small bubbles, while the translucent section contains relatively few large bubbles. This observation reveals the significance of time as well as pressure in relation to translucency of the finished product. Accordingly, the experiments reported in this paper were conducted to determine the effects of varying both intensity and duration of pressure upon translucency and associated bubble size and number. A third variable, durum variety, was introduced because of the importance of color characteristics in breeding new varieties.

Materials and Methods

The semolinas used in this study represented six varieties of durum grown in the 1943 wheat variety field plots at Langdon, North Dakota. They ranked from good to poor in quality in the following order: Carleton, Mindum, Stewart, Ld. 153, Kubanka-314, and Monad.

In the experimental method of processing tube macaroni (Fifield, 1934; Binnington and Geddes, 1936; and Harris and Sibbitt, 1942) it is not feasible to vary pressure because the dough is constantly changing in volume. Furthermore, the shape of conventional macaroni products is not suitable for objective measurements of light transmission and the true internal structure is masked by the roughened surface caused by friction with the die. On the other hand, the macaroni disc method of Fifield *et al.*, 1937 (the "micro test" of Cunningham and Anderson, 1943) is much more suitable for experimental study of the fundamental factors involved in macaroni manufacture. Any range of pressure or time can be applied, dough volume is approximately constant, the thin disc is convenient for measurements of color characteristics or light transmission, and the surface is not modified by friction.

Equipment available for these experiments was similar to that described by Fifield *et al.* (1937). Procedure in general was as follows: 30 g of semolina was used with 30% absorption and 120 revolutions of the mixer, followed by sheeting 30 times. Two discs were pressed from each sample. Drying was judged complete after two days between smooth faces of corrugated paper. Four pressures, 1,250, 1,500, 2,000, and 3,000 pounds per square inch for time periods of 20, 60, and 240 seconds, were chosen after preliminary experiments to determine what combinations would give the desired ranges in translucency. A lower range gauge would have been preferable to give more sensitive readings at still lower pressures, but at the time the work was undertaken only the 10-ton gauge was available.

Bubble numbers and diameters were determined under a microscope with an eyepiece micrometer at magnifications ranging from 17 to 430

diameters, as required by the various sizes of bubbles. Ten readings were made for each of the twin discs. Bubble counts were calculated to the number per 10 squares in the eyepiece reticule at 17 ×, which actually represented 6.94 square millimeters. Under the microscope it was possible to take bubble counts only in two dimensions. The square root of the number thus counted corresponds to the cube root of the number present in three dimensions. To facilitate graphing the data, bubble number was tabulated and shown in terms of the cube root of the number present in three dimensions. Diameter of bubbles was calculated to microns. Translucency of the discs, or the amount

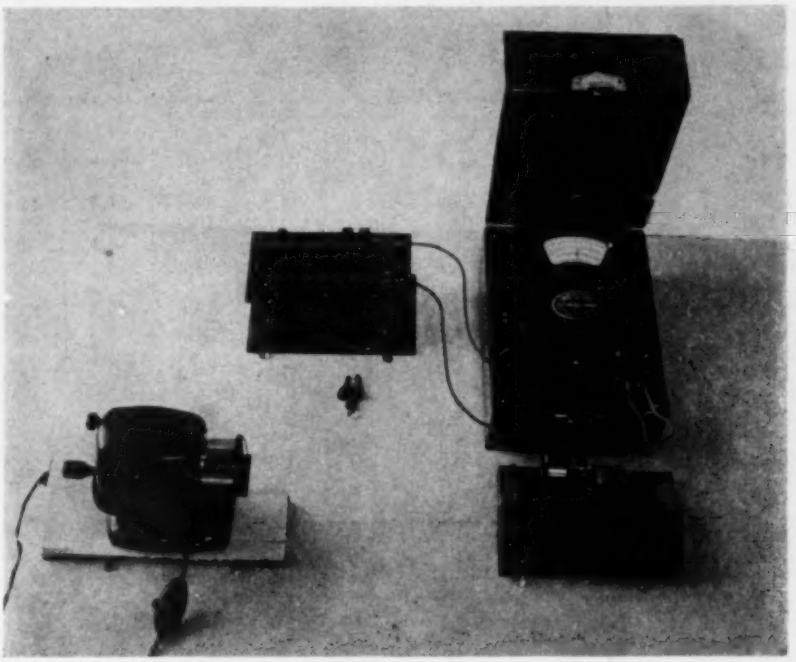


Fig. 1. Photo-electric cell assembly for measuring light transmission of macaroni discs.

of transmitted light that could pass through them, was measured with a photo-electric cell in terms of galvanometer scale units. The photo-electric cell assembly is illustrated in Figure 1. It consisted of a photronic cell wired to a galvanometer with a resistance box connected in parallel. The cell was placed facing the light source at a distance of 12 inches. The aperture of the cell was smaller than the discs to be measured. The most translucent macaroni disc was first held over the cell, and the resistance adjusted to give a maximum reading on the galvanometer. All discs were then read at this resistance. The light source was a small laboratory spotlight using a 100 watt bulb.

Experimental Results

Increasing duration and intensity of pressure on the macaroni discs caused the minute air bubbles to coalesce, reducing their number, thus increasing their size and augmenting the translucency of the discs.

Number of Bubbles. Table I presents data on the (cube root of) average bubble number for the six varieties at the various pressure and time period treatments.

TABLE I

EFFECT OF PRESSURE APPLIED FOR VARIOUS TIMES ON AVERAGE BUBBLE NUMBERS

Pressure	Time	Average bubble numbers [1]						
		Carleton	Ld. 153	Mindum	Stewart	Monad	Kubanka	Average
lb	*sec*							
none	none	77.8	67.4	70.4	70.4	66.1	65.6	69.6
1,250	20	23.0	23.9	23.5	23.2	24.2	23.5	23.6
1,500	20	9.7	10.0	10.1	9.6	10.2	9.7	9.7
2,000	20	4.7	4.9	4.6	4.7	4.7	4.6	4.7
3,000	20	3.1	3.5	2.7	3.0	3.4	3.1	3.1
1,250	60	14.1	13.9	12.8	14.3	13.8	13.5	13.7
1,500	60	4.6	5.7	4.3	5.1	4.5	4.3	4.8
2,000	60	3.5	3.9	3.6	3.6	3.2	3.4	3.5
3,000	60	2.4	2.8	2.9	2.7	2.3	2.3	2.6
1,250	240	4.0	5.1	4.4	4.0	3.8	3.4	4.1
1,500	240	3.2	3.4	3.2	3.0	3.0	2.6	3.1
2,000	240	2.9	2.9	3.0	2.7	2.3	2.7	2.8
3,000	240	2.1	2.2	2.4	2.1	2.1	1.7	2.1
Varietal averages		11.7	11.5	11.4	11.4	11.0	10.8	11.3

[1] Bubble numbers are expressed as cube root of average number determined in a disc volume of 18.5 cubic millimeters, 10 counts in each of twin discs for each variety treatment.

The relation between average number of bubbles and the pressure applied to the discs is curvilinear. The curves have been drawn to show an initial (dotted section) lag which was clearly evident when using pressures below 1,250 pounds as registered by a low pressure gauge in supplementary experiments. This is interpreted as the "yield value" (Gortner, 1938) characteristic of all plastic systems. After the lag period is overcome, the number of bubbles decreases greatly with relatively small increments of pressure. This raises the question whether the air bubbles are squeezed out, or merely coalesced into larger and fewer bubbles. Data presented later on the size of bubbles give no indication that any air is forced out of the dough.

The effect of pressure increases with the length of time it is applied, for example 2,000 pounds at 20 seconds, 1,500 pounds at one minute,

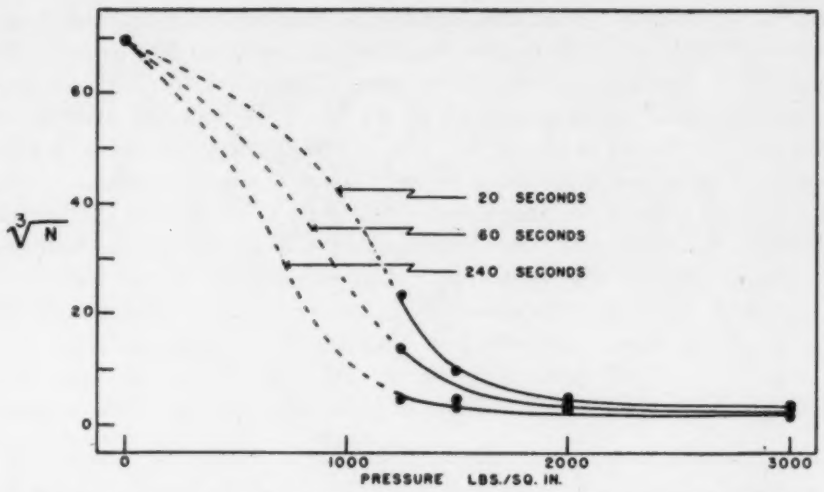


Fig. 2. Average number of air bubbles (expressed as the cube root) in macaroni discs made from six durum wheat varieties, employing various pressures for different time intervals. Under the microscope it was possible to take bubble counts in only two dimensions. The square root of the number thus counted corresponds to the cube root of the number present in three dimensions.

and 1,250 pounds at four minutes all gave approximately the same number of bubbles. The actual number of bubbles in a given volume in the unpressed check was nearly 40,000 times that in the disc receiving 3,000 pounds pressure for four minutes. Varietal differences in bubble number were small.

TABLE II

EFFECT OF PRESSURE APPLIED FOR VARIOUS TIMES ON AVERAGE DIAMETER OF AIR BUBBLES

Pressure	Time	Average bubble diameters						
		Ld. 153	Mindum	Carleton	Stewart	Monad	Kubanka	Average
lb	*sec*	μ	μ	μ	μ	μ	μ	μ
none	none	11	17	14	13	13	12	13.4
1,250	20	24	27	28	30	31	34	29.0
1,500	20	54	86	78	69	62	85	72.5
2,000	20	130	151	165	155	209	186	166.0
3,000	20	264	326	273	304	293	312	295.0
1,250	60	62	59	60	56	58	63	59.8
1,500	60	165	190	194	207	226	245	204.6
2,000	60	272	253	309	268	356	293	291.9
3,000	60	338	332	380	419	479	437	397.6
1,250	240	184	210	245	236	260	318	233.7
1,500	240	289	267	277	347	416	394	348.2
2,000	240	319	323	341	400	425	401	368.4
3,000	240	334	421	417	522	611	673	496.2
Varietal averages		203	220	231	251	286	295	247.6

Size of Bubbles. Macaroni bubbles in commercial macaroni products are highly variable in size, but often range from 200 to 400 μ in diameter. Average bubble diameters in microns for the various treatments and varieties are given in Table II and the treatment averages are shown in Figure 3. The initial lag period is more clearly indicated by these data than in the case of bubble numbers. The effect of duration of pressure is more strikingly illustrated at the lowest pressure, 1,250 pounds. At 20 seconds the diameter of the bubbles was barely double that of the check, at one minute it had increased four times, but at four minutes the diameter had increased nearly 20 times. Time was relatively less important as pressures were increased. At 3,000 pounds pressure, 20 seconds of pressing was sufficient to produce larger bubbles than four minutes at 1,250 pounds.

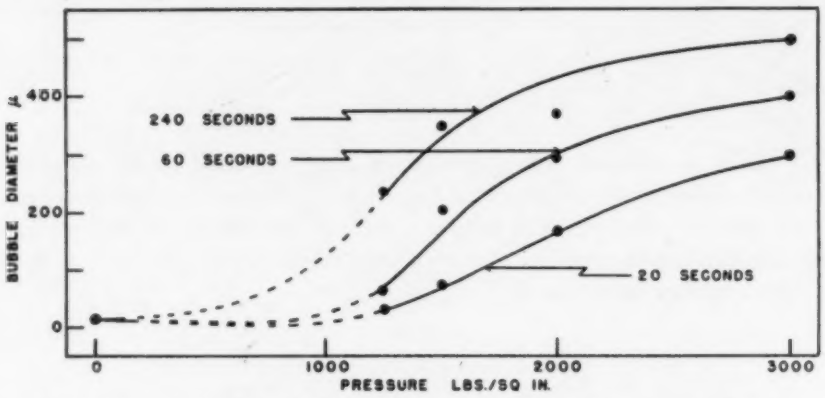


Fig. 3. Average size of bubbles in macaroni discs made from six durum wheat varieties, employing various pressures for different time intervals.

In the case of bubble size, the curves had not leveled off at 3,000 pounds of pressure for four minutes. Although this treatment gave bubbles 40 times as large in diameter as the checks, it appears that either higher pressures or longer time periods would have given still larger bubbles.

Photomicrographs showing the sizes of bubbles at four selected treatments are given in Figure 4.

The effect of duration of pressure on macaroni products is less obvious than the effect of intensity of pressure alone. It was previously mentioned that the first macaroni pressed out of the die is opaque, but that subsequent portions gradually become translucent. Actual measurements of size of bubbles in such a tube of macaroni gave as an average of 10 determinations each, 65 $\pm$ 0.08 μ for the opaque section, and 309 $\pm$ 0.21 μ for the translucent section. Both sections of the macaroni tube had received pressure sufficient to force them through

the die, but the high pressure had not been acting on the opaque section long enough to make it translucent.

Light Transmission of Discs. Light transmission of the various discs is given in Table III and Figure 5. The curves resemble those for bubble number and bubble size. First there is a definite lag period, then a rapid increase in light transmission at the same treatments which gave rapid changes in bubble numbers and bubble size. The light transmission curves tend to level off more rapidly than did those

Fig. 4. Photomicrographs showing relative size of bubbles in selected macaroni discs: (1) 1,500 pounds for 20 seconds; (2) 3,000 pounds for 20 seconds; (3) 1,500 pounds for 240 seconds; (4) 3,000 pounds for 240 seconds.

representing bubble size, presumably toward a value determined by the index of transmission of the pure macaroni without any bubbles.

Changes in light transmission were very striking. The most severe treatment permitted the passage of seven times as much light as the unpressed checks. These values give quantitative expression to the observation that "translucency is a result of pressure."

Analyses of Variance. The variance analyses are summarized in Table IV for the different variables investigated. Values determined

TABLE III

EFFECT OF PRESSURE APPLIED FOR VARIOUS TIMES ON AVERAGE LIGHT TRANSMISSION OF MACARONI DISCS

Pressure	Time	Light transmission values [1]						
		Ld. 153	Monad	Mindum	Kubanka	Stewart	Carleton	Average
lb	*sec*							
none	none	3.2	2.9	2.6	4.9	2.8	4.5	3.4
1,250	20	7.6	7.4	6.9	9.1	7.3	7.1	7.6
1,500	20	10.6	11.0	12.4	9.7	11.3	10.1	10.9
2,000	20	16.6	17.4	18.2	17.9	18.0	17.7	17.6
3,000	20	19.4	19.8	20.5	19.8	20.7	21.2	20.2
1,250	60	11.6	11.1	10.3	11.3	11.2	11.9	11.2
1,500	60	17.2	16.7	18.5	17.1	17.2	18.4	17.5
2,000	60	20.8	20.1	19.5	20.8	20.5	22.4	20.5
3,000	60	21.8	22.4	22.6	22.5	21.6	23.7	22.4
1,250	240	18.8	18.9	19.4	19.2	19.2	20.1	19.3
1,500	240	20.1	20.5	21.0	20.9	22.5	20.6	20.9
2,000	240	20.7	21.5	21.9	21.8	23.3	22.0	21.9
3,000	240	22.4	24.4	23.3	22.7	24.1	24.6	23.6
Varietal averages		16.2	16.5	16.7	16.7	16.9	17.2	16.7

[1] Light transmission measured in galvanometer scale units at 1.7 ohms resistance.

for twin discs at each treatment entered separately into the calculations, to give a basis for evaluating experimental error. Data for the unpressed checks were not included in the analyses.

In the case of bubble numbers, both time and pressure, and their interaction, made highly significant contributions to variance, but

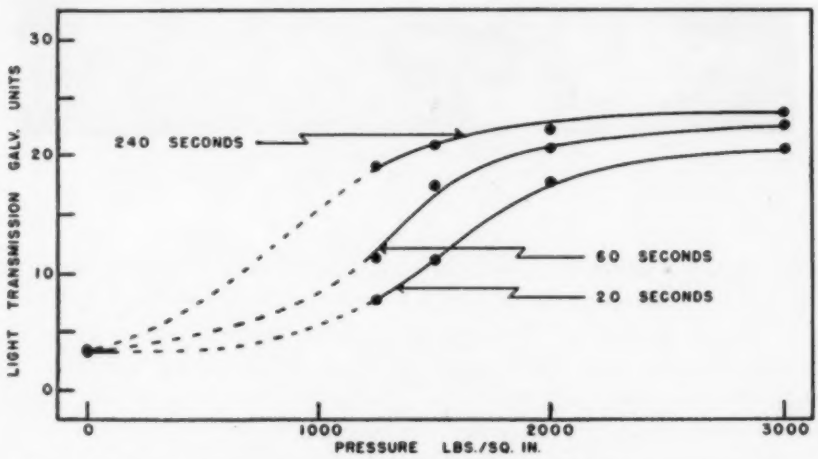


Fig. 5. Average light transmission of macaroni discs made from six durum wheat varieties, employing various pressures for different time intervals.

TABLE IV

VARIANCE ANALYSES OF BUBBLE NUMBER, BUBBLE SIZE, AND LIGHT TRANSMISSION

(Data for unpressed checks not included)

Variance due to	Degrees of freedom	Mean squares for		
		Bubble number	Bubble size	Transmission
Varieties	5	4.0	32,324 **	31.0 **
Time	2	833.5 **	599,793 **	6,479.0 **
Pressure	3	1,126.7 **	519,456 **	6,164.0 **
Varieties × time	10	3.7	10,042 *	16.5 *
Varieties × pressure	15	2.5	3,497 *	13.1 *
Time × pressure	6	210.3 **	11,284 **	631.7 **
Error (V × T × P)	30	1.9	1,519	6.7
Total (Treatments)	71			
Twins (Within treatments)	72	0.3	89	0.9
Total	143			

* Significance exceeds 5% point.
** Significance exceeds 1% point.

neither varietal differences nor the interactions involving variety showed statistical significance.

All three variables, and their first order interactions, contributed very significantly to the variability of both bubble size and light transmission, although variety was consistently least important.

Analysis of the variance in bubble number, bubble size, and light transmission values clearly showed in each case that the time and pressure treatments were mainly responsible for the differences.

Discussion

If no air is forced out, one would expect the number of bubbles to vary inversely with the cube of the diameter. In Figure 6, bubble diameter is plotted against bubble number for each treatment. This gives a hyperbolic curve characteristic of the reciprocal relation, expressed by the equation

$$xy = a$$

where a is a constant, x is the diameter of the bubbles, and y the cube root of bubble number. The data give a good fit to the theoretical curve for this function.

Using logarithms of x and y, the relation may be transformed to a straight line. Figure 6 shows that the fit of the transformed data to the straight line is very good.

Size of bubbles at the various treatments, therefore, corresponds closely with the size calculated from the corresponding bubble numbers.

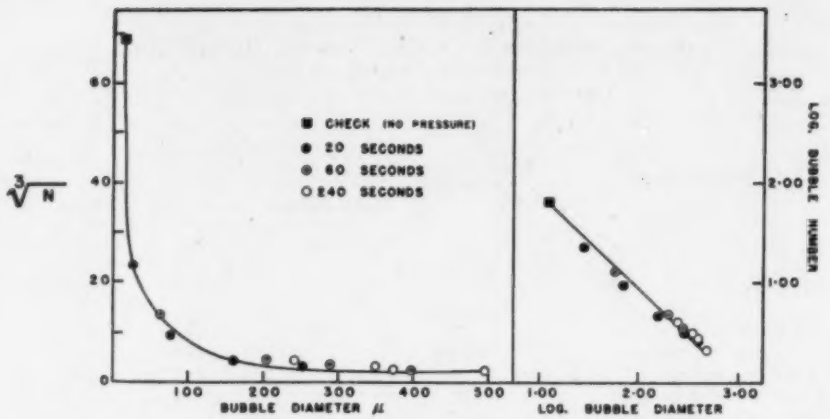


Fig. 6. The relation of bubble diameter to cube root of bubble number ($\rho_{xy} = 0.96$), and of log bubble diameter to log bubble number in macaroni discs made with varying pressures applied for different time intervals ($r_{xy} = -0.99$).

Plotting light transmission of the discs against the corresponding bubble size gave the graph shown in Figure 7. The plotted points follow a curvilinear path, resembling a logarithmic curve. Plotting light transmission against the logarithm of the bubble diameter gave the straight line shown in Figure 7. By inspection, the following formula for the straight line was derived:

$$Y = 13.8 \log x - 13.8$$

where Y is light transmission and x is bubble diameter. Transforming $\log x$ to the antilog, the theoretical curve was then drawn, and it fitted the original plotted points very satisfactorily.

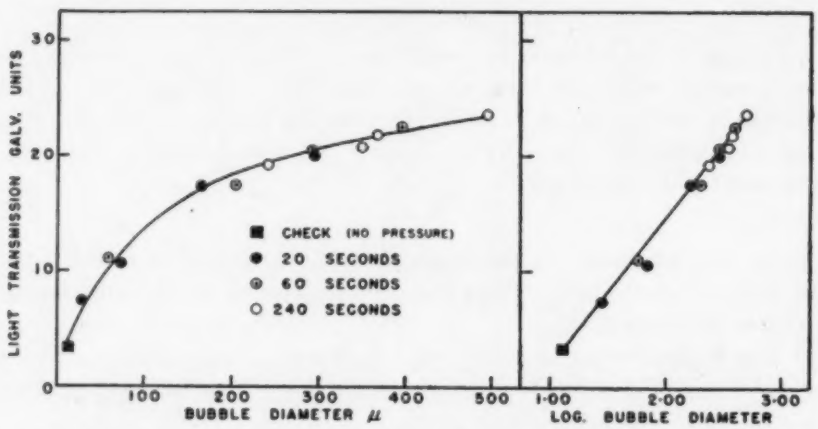


Fig. 7. The relation of light transmission to bubble diameter, and to log bubble diameter in macaroni discs made with varying pressures applied for different times.

The data did not fit a parabolic curve of the type:

$$y^2 = 2\,px.$$

The above curve does not represent the inherent effect of bubble size alone upon light transmission. When dry macaroni products or macaroni discs are held up to the light, the bubbles are apparent as shadows. Therefore, increasing bubble diameter, of itself, would reduce transmission, were it not for the simultaneous decrease in bubble number. Bubble number changes inversely as the cube of the diameter and the curve indicates that the net change in transmission is in the direction of change in number. In this experiment, bubble diameters increased 40 times when the maximum pressure was applied, but simultaneously bubble numbers were reduced 40,000 times, so translucency is affected most by the latter. The curve represents the resultant effect of the two opposing influences, bubble size and bubble number, in a closed system where decrease in number involves an increase in size.

Variations in light transmission were plotted against bubble numbers also, but it was more difficult to reduce this curve to a mathematical formula; hence, it is not presented here. The same interacting effects are involved as discussed above. Light entering the unpressed disc is so diffused by the numerous bubbles that most of it cannot pass through; whereas, the light passes through the pressed discs with only slight interruption by the few bubbles, even though these are relatively large.

One may speculate as to what causes the bubbles to coalesce under pressure. Theoretical considerations suggest that in coalescing, the bubbles are reducing their total surface area exposed to pressure. Eight bubbles, each of one unit diameter, would reduce their total surface area one-half by coalescing into a single bubble of two units diameter. Bubbles within the plastic dough are more or less free to move, and in coalescing they reduce the total pressure on the enclosed air.

However, this appears to be a result rather than the cause of coalescing. As a cause, it is suggested that the compressible air bubbles interfere with the equal distribution of the applied pressure throughout the dough. If two bubbles are pictured as near to each other, the pressure on all sides can be assumed to be at a maximum, except on their adjacent sides, where it would be mutually reduced. The two bubbles about to coalesce in Figure 4(2) seem to offer graphic support of this theory. If the pressure on adjacent sides is thus reduced, the bubbles will move toward each other at an accelerating rate and coalesce.

Such differences in translucency as were observed were explainable in terms of bubble differences, and could not be related to yellow pigment, or amount of gray or red coloration. This indicates that light transmission readings alone are not suitable for evaluating the macaroni color of new durum hybrid selections as has been intimated in the literature.

Apparently no air was forced out of the macaroni discs used in these experiments. The air simply was coalesced into larger bubbles. Probably the same thing happens in the commercial macaroni process, although these experiments were not designed to test that point. It did seem that most macaroni products examined contained less air than these experimental discs. If this be true, it may be due to differences in mixers, or in mixing time. The small experimental mixer used here had small round pins which may incorporate more air into the dough than the flat paddle-shaped mixers used commercially.

However, the general implications of this study should be applicable to commercial processing. Translucency in macaroni products depends primarily upon pressure, both its intensity and its duration. Pressure produces translucency by coalescing the air bubbles. Small scale "slab tests" made without the application of pressure will necessarily give an opaque product.

Summary

Macaroni discs which had received no pressure contained great numbers of air bubbles less than 20 μ in diameter, and were very opaque. Application of 3,000 pounds pressure per square inch for four minutes coalesced these bubbles, giving translucent discs with bubbles 40 times as large in diameter, and reducing the number nearly 40,000 times. Light transmission increased six times. Intermediate treatments gave intermediate effects, but all results showed curvilinear relations.

Following application of pressure, there was a lag period before significant changes in bubble size or number were evident. This was interpreted as the "yield value" characteristic of plastic systems. At lower pressures the time factor was a very important influence. The first strands of macaroni pressed from a batch of dough are opaque because the pressure has not been operating for sufficient time. Laboratory "slab tests" which omit pressure will not give a translucent product.

Varietal differences in six widely different varieties were not striking. Monad and Kubanka gave somewhat larger bubbles and higher light transmission at the more severe treatments. Varietal differences

in light transmission could not be related to differences in yellow pigment, nor to undesirable red or gray colors.

Light transmission, a measure of translucency, is the resultant of two opposing and interdependent forces, decreasing bubble numbers and increasing bubble size. Because of its mathematical relation to size, bubble number changes faster than size; and therefore translucency increases as bubble number decreases, despite simultaneous increases in bubble size.

Pressure increases translucency by causing the bubbles to coalesce. A hypothesis is offered to explain why the bubbles coalesce.

Acknowledgment

The authors wish to acknowledge the assistance of J. Harwood Longwell in securing the data on bubble diameters.

Literature Cited

Alsberg, C. L.
1939 Durum wheats and their utilization. Wheat Studies of the Food Research Inst. **15** (7): 337–364.
Binnington, D. S., and Geddes, W. F.
1936 Experimental durum milling and macaroni making technique. Cereal Chem. **13**: 497–521.
Cunningham, R. L., and Anderson, J. A.
1943 Micro tests of alimentary pastes. I. Apparatus and method. Cereal Chem. **20**: 171–185.
1943a Micro tests of alimentary pastes. II. Effects of processing conditions on paste properties. Cereal Chem. **20**: 482–506.
Fifield, C. C.
1934 Experimental equipment for the manufacture of alimentary pastes. Cereal Chem. **11**: 330–334.
——, Smith, G. S., and Hayes, J. F.
1937 Quality in durum wheats and a method for testing small samples. Cereal Chem. **14**: 661–673.
Gortner, R. A.
1938 Outlines of Biochemistry. 1017 pp. John Wiley and Sons, New York.
Harris, R. H., and Sibbitt, L. D.
1942 Experimental durum milling and processing equipment, with further quality studies on North Dakota durum wheats. Cereal Chem. **19**: 388–402.

A MICRO-MILLING TECHNIQUE USING THE HOBART GRINDER [1]

KARL F. FINNEY and WILLIAM T. YAMAZAKI [2]

(Received for publication April 25, 1946)

Several workers including Johnson and Swanson (1942), McCluggage (1943), Lamb (1944), and Finney and Yamazaki (1946) have used a Hobart or some other type of grinder for obtaining flour for testing and evaluating the quality characteristics of small samples of commercially and experimentally grown wheat varieties. These flours have been comparatively high in ash content, however, due to relatively large quantities of finely divided bran particles. Considerable work with 100-g wheat samples has been carried out in the Federal Soft Wheat Laboratory in an effort to reduce the ash of flour obtained in this manner. This paper describes a micro-milling procedure involving two or three preliminary breaks through the rolls of the Tag-Heppenstall moisture meter and one break and two reductions on a Hobart grinder, and presents data showing the techniques responsible for obtaining flours having ash and protein contents comparable to those obtained by the usual experimental milling procedures.

Materials

Two wheat composites, one hard and the other soft red winter, were used in the preliminary work which established the amount and time of temper, Hobart grinder settings, number and size of sieves, sifting time, and other important techniques constituting the milling procedure. The hard winter composite consisted of seven varieties from each of four locations in the central and southern great plains. The soft winter composite consisted of 43 samples representing several varieties and locations in the soft winter wheat area.

The procedure described herein was applied to about 300 additional individual samples of soft red winter, white, hard red winter, and hard red spring wheat varieties from representative locations in the entire wheat-producing area of the United States, except the Pacific Coast.

Method

Previous to milling on the Hobart grinder, each cleaned 100-g wheat sample was run through the Tag-Heppenstall moisture meter

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associate Chemist and Junior Chemist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

(first preliminary break). On the basis of the wheat moisture, each Tag-broken sample of hard winter and spring wheat was given a first temper to 14% moisture for 48 to 72 hours. Soft winter wheats were tempered to 12.5%. Limited data, however, have indicated that a first temper time of 18 to 24 hours was sufficient. A second temper of 2% was added to all samples 45 to 60 minutes before milling. Each sample was again run through the Tag-moisture meter one to three times (additional preliminary breaks) to shatter the kernels completely,

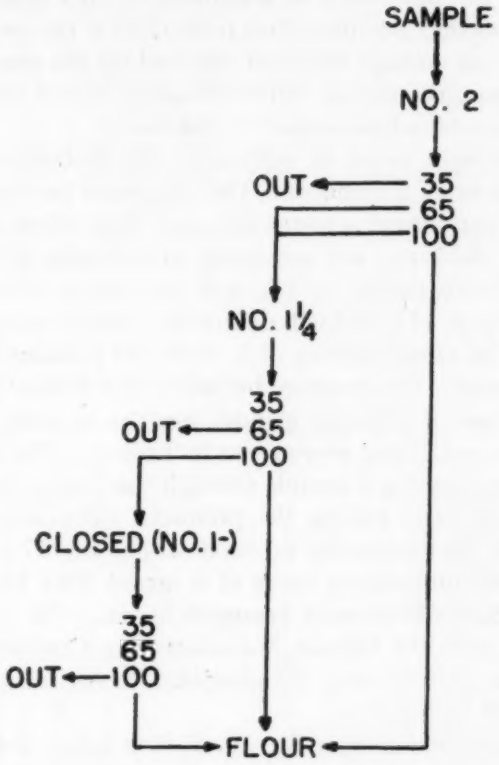


Fig. 1. Milling flow sheet for obtaining low-ash flours using a Hobart grinder.

after which it was subjected to one break and two reductions on the Hobart grinder with three siftings on a Ro-Tap. The relatively simple flow sheet showing Tyler sieves (meshes per inch) and grinder settings (Nos. 2, 1¼, and closed or 1 −) is shown in Figure 1.

It is extremely important that the wheat and middlings be fed slowly. For 100-g samples the wheat-feed time was about 45 to 60 seconds. The middlings were fed slowly so as not to overload the grinder as indicated by the sound of the motor and burrs. In each of the two reductions, the middlings were run through a second time to

push through middlings which had lodged at any point. The sifting times for hard winters and springs were usually 1, 2, and 2 and for soft red winters and whites 2, 3, and 3 minutes for the first, second, and third siftings, respectively. In all instances, however, the bran was weighed at ½-minute intervals and was usually discarded after ½ to 1 minute. This was, as a rule, sufficient time to give a bran weight of 29 to 31 g. Bran weights appreciably greater than 31 g after 1 minute of sifting usually contained middlings, most of which passed through the sieve after an additional ½ to 1 minute of sifting.

During the second grinding (first reduction) if the overs of the 65-mesh sieve are run through first and followed by the overs of the 100-mesh sieve, a finer granulation will be obtained than if the overs of the two sieves are combined previous to reduction.

Another pertinent point in milling on the Hobart grinder is the proper adjustment of the burrs. The distances between the burrs for the 2, 1¼, and closed settings are such that when the grinder is running empty the burrs will not touch at a setting of 1⅜, will not touch or touch very faintly at 1¼, will just touch at several points over their surface at 1⅛, and will distinctly touch at many points at a setting of 1. The closed setting of 1 − should produce a slight howl or screeching sound. Other sets of burrs or other Hobart grinders may necessitate the use of different grinder settings in order to maintain the approximate weights of overs given in Table I. The average total time required for running a sample through the Tag, grinding, sifting, weighing, labeling, and mixing the products, calculating flour yield, and cleaning out the grinder for the next sample was 17 to 20 minutes.

The left hand pulverizing burrs of a model 1044 Hobart grinder were used for the milling work reported herein. As a result of co-operative work with the Hobart Manufacturing Company, the burrs (both rotor and stator) were standardized to an average depth of openings of 0.004 inches.

When a 2% second temper was used, *hard wheat* flours contained about 15% moisture. To insure safer storage, their moisture contents were reduced to about 14% by drying for 2 hours in a forced air oven held at room temperature after simply removing their ½ pint-container caps.

Experimental Results

The factors of tempering, wheat feed time, and amount of preliminary breaking or shattering previous to breaking the wheat in the Hobart grinder are extremely important in obtaining low ash flours. The data relative to these techniques are given in Table I and Figure 2.

The importance of the amount of temper is readily seen from the milling and analytical data for the hard winter composite shown in

TABLE I

MILLING AND CHEMICAL DATA FOR FLOURS MILLED ON THE HOBART GRINDER FROM 100-G SAMPLES OF A HARD RED WINTER WHEAT COMPOSITE TEMPERED TO DIFFERENT MOISTURE CONTENTS

Wheat moisture after 1st temper	Water added for 2nd temper	1st break feed [1] time	Overs 35 mesh sieve 1st sifting	Overs 65 mesh sieve		Overs 100 mesh sieve 3rd sifting	Flour		
				2nd sifting	3rd sifting		Yield	Moisture	Ash [2]
%	%	sec	g	g	g	g	g	%	%
11.0	0	58	24.7	3.26	0.79	1.97	65.8	11.5	.57
11.5	0	58	24.7	3.15	0.76	1.71	67.0	11.7	.55
12.0	0	57	25.1	3.42	0.84	1.82	67.4	12.0	.55
12.5	0	58	24.9	2.86	0.68	1.51	69.5	12.3	.54
13.0	0	58	25.5	3.07	0.78	1.49	68.7	12.7	.52
13.5	0	60	26.2	2.65	0.64	1.69	68.7	13.0	.50
14.0	0	59	26.3	2.77	0.63	1.52	69.0	13.3	.49
14.5	0	60	27.5	2.59	1.13	1.65	68.6	13.8	.48
13.0	2	60	28.9	2.88	0.70	1.41	66.4	13.6 [3]	.47
13.5	2	60	29.7	2.84	0.81	2.26	66.3	13.6 [3]	.45
14.0	2	60	30.3	2.74	1.13	2.26	66.2	13.9 [3]	.45
14.5	2	64	31.2	2.92	1.31	2.44	65.4	13.9 [3]	.45

[1] All samples received 3 preliminary breaks.
[2] 14% moisture basis.
[3] Dried for 2 hours in a forced air oven held at room temperature.

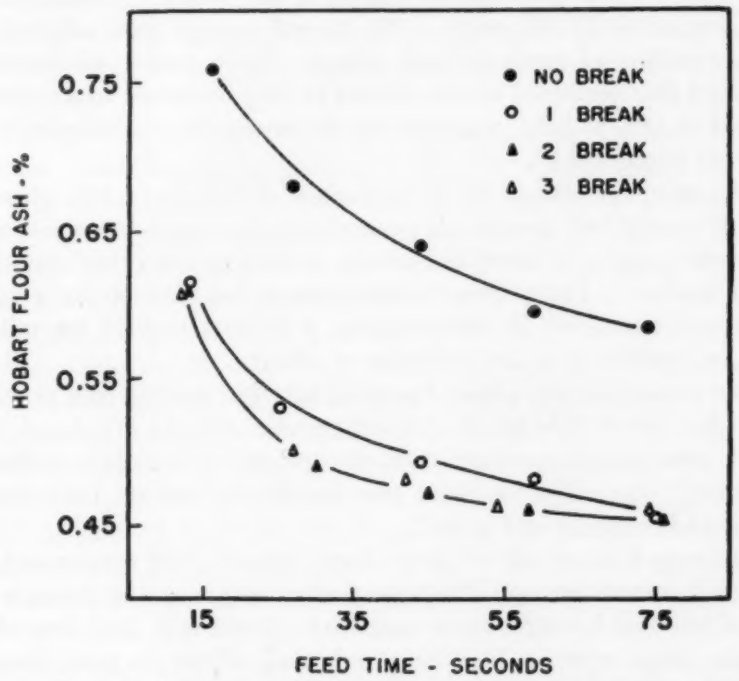


Fig. 2. Effect of preliminary breaking through the Tag-Heppenstall moisture meter and first-break feed time on the flour ash of 100-g wheat samples milled on the Hobart grinder.

Table I. As the moisture content of the grain was increased from 11% to 14.5% by varying the amounts of first temper water, the flour ash decreased from 0.57% to 0.48%. A second temper of 2% resulted in a further reduction in ash. For example, 2% second temper in combination with a first temper to 13 and 13.5% lowered the ash 0.05%. The second temper was somewhat less effective, however, when used in conjunction with a first temper to 14% and 14.5% moisture. The flour yields, which averaged about 68% when using only a first temper, were about 1.5 to 2% lower when a second temper also was used. The reduction in ash with the second temper, however, appears to more than compensate for the reduction in flour yields.

As the moisture added at the first temper was increased, the weight of overs on the 35-mesh sieve increased. The addition of 2% more water as a second temper resulted in additional and consistent increases. When using the 14.5 plus 2% second temper, however, there was a slight tendency for the flour to cake on the inside walls of the grinder. As a result, a first temper to 14% was used in conjunction with a 2% second temper in all routine work on hard wheats.

In a similar series for soft wheat, however, a first temper to more than 12.5% moisture with a 2% second temper resulted in caking on the walls of the grinder and a clogging of the sieves. Accordingly, a first temper to 12.5% with a 2% second temper was adopted for routine milling of typically soft wheat. More recent studies have indicated that semihard wheats should be first tempered to a moisture content of 13% to 14%; whereas, for the extremely soft samples, 12% moisture is preferable.

The weights of the overs of the various sieves for the 14% plus 2% second temper are typical, in general, of the results obtained with numerous samples of hard red winter as well as the other classes of wheat studied. Thus, these weights should be valuable as a guide to anyone interested in standardizing a Hobart grinder for milling small or possibly even large samples of wheat.

The amount of preliminary breaking between the Tag rolls previous to the first break through the Hobart grinder, and the first-break feed time is even more important than the amount of temper in reducing flour ash. The effect of these two factors on Hobart flour ash is strikingly illustrated in Figure 2.

All flours represented in Figure 2 were given a first temper to 14% and a 2% second temper. With no preliminary breaking through the rolls of the Tag moisture meter and with a first-break feed time of 16 seconds (time required for 100 g unbroken wheat to pass through grinder), a relatively high flour ash of 0.76% was obtained. Flour ash,

however, decreased from 0.76% to 0.58% as the feed time was increased from 16 to 74 seconds.

Putting samples once through the Tag previous to the first break on the Hobart grinder and using a first-break feed time of 13 seconds lowered the flour ash from 0.76% to 0.62%, a reduction comparable to that obtained with no preliminary breaking and a long feed time of 59 seconds. Using one preliminary break, the flour ash was reduced from 0.62% to 0.45% with increasing feed time. Thus, at a given feed time, one break through the Tag rolls reduced the flour ash approximately 0.16%. The flour ashes resulting from the use of two and three Tag breaks when plotted against feed time define a single curve which is only slightly below that for one preliminary break, indicating that more than two breaks are unnecessary. Thus, it appears that for a normal hard wheat, such as is represented by the hard winter composite, two preliminary Tag breaks and a feed time of 1 minute will give a minimum ash content. It has been found, however, that for shriveled samples of wheat, as many as three or four Tag breaks were desirable. For example, Hobart flours having ash contents of 0.57%, 0.55%, 0.53%, and 0.52% were obtained from an appreciably shriveled, high-protein hard winter wheat as a result of the use of one, two, three, and four preliminary breaks, respectively. Thus, there is the probability of a lower ash to be gained and only a few seconds to be lost by passing the sample through the Tag rolls an extra time or two.

The Hobart milling procedure described herein has been applied to more than 300 samples (100-g) of soft red winter, white, hard red winter, and hard red spring wheats grown throughout the entire wheat-producing area of the United States, except the Pacific Coast. Typical milling and analytical data for representative samples of these different classes of wheat are shown in Table II.

Within the group of soft red winter and white wheat flours from Wooster, Ohio, and East Lansing, Michigan, the Hobart flour ashes varied from 0.43% to 0.53%, half of them being 0.49% or lower. Within the group of hard red winters, the flour ashes varied from 0.47% to 0.54%, being somewhat higher than for the soft wheat flours. The flours from the hard red spring wheats grown at Bozeman, Montana, however, were appreciably higher in ash than either the soft or hard winter flours; whereas, those from Langdon, North Dakota, averaged slightly lower in ash content than the soft wheat flours.

The protein contents of the Hobart flours were lower than those of the wheats by 0.5% to somewhat over 1%, and in this respect are comparable to Buhler and Allis straight grade flours. The flour yields

TABLE II

CHEMICAL AND MILLING DATA FOR DIFFERENT CLASSES AND VARIETIES OF WHEAT GROWN AT SEVERAL LOCATIONS AND FOR FLOURS MILLED FROM THEM ON THE HOBART GRINDER

Variety and location	Wheat		Flour			
	Ash[1] %	Protein[1] %	Moisture %	Ash[1] %	Protein[1] %	Yield %
SOFT RED WINTER AND WHITE						
Wooster, Ohio						
Clarkan	1.87	12.3	14.3	.49	11.1	65.5
Trumbull	1.80	11.5	14.3	.51	10.4	66.0
Fairfield	1.74	11.2	14.3	.50	9.8	65.3
Thorne	1.81	12.6	14.4	.48	11.4	64.5
Wabash	1.78	12.2	14.4	.43	11.0	65.4
Blackhawk	1.80	12.5	14.4	.46	11.5	68.3
Am. Banner	1.80	11.9	14.3	.53	10.9	66.4
E. Lansing, Mich.						
Clarkan	1.80	9.3	14.2	.52	8.5	64.7
Trumbull	1.71	9.0	14.1	.49	8.1	66.9
Fairfield	1.69	8.7	14.2	.53	7.5	64.6
Thorne	1.74	9.1	14.1	.53	8.3	64.8
Wabash	1.65	9.0	14.4	.47	8.2	66.2
Blackhawk	1.75	10.3	14.4	.49	9.0	67.5
Am. Banner	1.71	9.2	14.2	.52	8.2	66.9
Composite of several varieties and stations						
S.R.W. Std.	1.73	10.6	14.2	.45	9.5	65.7
HARD RED WINTER						
Central district composites						
Kharkof	1.88	14.8	13.9	.51	13.5	62.5
Blackhull	1.83	14.3	14.4	.52	13.4	65.9
Tenmarq	1.83	14.2	14.0	.50	13.0	64.6
Early B. H.	1.75	13.1	14.2	.49	12.4	66.4
Pawnee	1.62	14.3	14.7	.51	13.2	65.5
Comanche	1.73	14.5	14.7	.51	13.8	64.5
Chiefkan	1.75	13.8	14.2	.54	13.2	65.2
Wichita	1.72	14.1	14.3	.53	13.5	66.4
Woodward, Okla.						
Kharkof	1.51	9.8	14.8	.52	8.9	67.3
Blackhull	1.35	9.7	14.9	.51	8.5	67.3
Tenmarq	1.32	10.5	14.5	.53	9.6	68.1
Early B. H.	1.58	11.3	14.3	.47	10.6	65.4
Pawnee	1.38	10.3	14.5	.52	9.5	66.5
Comanche	1.47	11.0	14.6	.50	10.3	67.4
Chiefkan	1.45	10.5	14.6	.50	9.7	67.4
Wichita	1.48	10.9	14.5	.51	10.1	64.7
Composite of several varieties and stations						
H.R.W. Std.	1.45	13.6	14.5	.45	13.0	67.7

TABLE II (Continued)

Variety and location	Wheat		Flour			
	Ash [1] %	Protein [1] %	Moisture %	Ash [1] %	Protein [1] %	Yield g
HARD RED SPRING						
Bozeman, Mont.						
Thatcher	1.56	11.8	14.7	.54	11.3	67.1
Pilot	1.66	11.5	14.7	.52	10.9	62.5
Mida	1.75	11.2	15.5	.55	10.7	64.4
Cadet	1.65	13.1	14.8	.59	12.8	63.6
Newthatch	1.65	12.3	15.2	.53	12.1	68.1
Marquis	1.68	12.9	14.9	.57	12.6	63.0
Ceres	1.70	11.8	14.8	.57	11.2	63.8
Langdon, N. D.						
Thatcher	1.66	14.3	14.1	.51	13.6	63.3
Pilot	1.65	14.3	14.1	.46	13.2	60.5
Mida	1.55	14.4	14.2	.48	14.0	63.6
Cadet	1.54	14.4	14.2	.46	14.2	63.6
Newthatch	1.66	15.3	14.5	.49	15.2	66.4
Rival	1.58	14.6	14.7	.53	14.2	67.8
Regent	1.56	15.0	14.3	.46	14.7	65.9

[1] 14% moisture basis.

averaged about 65 g from somewhat over 100 g of tempered grain and appear to be satisfactory in view of the small quantity of wheat milled.

The data presented in Table II show conclusively that a straight-grade flour with ash and protein levels comparable to those expected on the Buhler and Allis experimental mills can be obtained by milling 100-g wheat samples on the Hobart grinder. The ash contents of the Hobart-milled flours are comparable to those reported by Geddes and Aitken (1935) for flours obtained on their miniature, Allis-type experimental mill.

Summary

A comparatively simple milling procedure involving one or two preliminary breaks through the rolls of the Tag-Heppenstall moisture meter and one break and two reductions on the Hobart Coffee grinder is described whereby straight grade flours having ash and protein contents approaching those expected on Buhler and Allis experimental mills can be obtained from 100-g wheat samples.

Proper tempering, preliminary breaking or shattering of the wheat, and first-break feed time were about equally responsible for the reduction in flour ash content from somewhat under 1% to 0.45%.

Using the procedure and technique described, the Hobart grinder is useful as a simple and inexpensive micro-flour mill.

Acknowledgment

Credit is due the Hobart Manufacturing Company for its cooperation in work concerned with the effect of size of burr openings on flour yield and ash content.

Literature Cited

Finney, K. F., and Yamazaki, W. T.
1946 Water retention capacity as an index of the loaf volume potentialities and protein quality of hard red winter wheats. Cereal Chem. **23**: 416–427.
Geddes, W. F., and Aitken, T. R.
1935 An experimental milling and baking technique requiring 100 grams wheat. Cereal Chem. **12**: 696–707.
Johnson, J. A., Jr., and Swanson, C. O.
1942 The testing of wheat quality by recording dough mixer curves obtained from sifted wheat meals. Cereal Chem. **19**: 216–229.
Lamb, C. A.
1944 Sifted wheat meal mixograms for selecting soft wheat varieties. Cereal Chem. **21**: 57–64.
McCluggage, M. E.
1943 Micro milling and baking of small samples of wheat. Cereal Chem. **20**: 185–193.

A STUDY OF SORGHUM AND SORGHUM STARCHES [1]

F. E. Horan [2] and Marie F. Heider

Midwest Research Institute, Kansas City, Missouri

(Received for publication May 23, 1946)

During the years of the war circumstances have made it extremely difficult for the manufacturers of starch, principally corn starch, to receive ample supplies of source materials. As a consequence, the wet-millers have been forced on a few occasions in recent years to resort to other starchy cereals, such as wheat and sorghum. New milling techniques have been developed by Slotter and Langford (1944) for the processing of wheat, owing to the unique doughing characteristics of the protein in this species of grain. The same procedures used with corn were applied to sorghum grains, but the results were not entirely satisfactory and undoubtedly many improvements will be added in connection with the processing of these latter grains.

Martin (1937) describes sorghum as a comparatively new crop in the United States, although it was cultivated as far back as 700 B.C. In the dry, hot, midwestern and southwestern states sorghum is greatly appreciated as a source of grain and forage for livestock. In general, sorghums are divided among several different groups that have distinct uses in this country. The grain sorghums, such as kafir, milo, hegari, feterita, and darso, are grown primarily for their grain. The sorgos (sweet sorghums or cane) are grown for the production of syrup or for forage.

[1] Presented before the Atlantic City meeting of the American Chemical Society, April 8–12, 1946.
[2] Corn Products Refining Company Research Fellow.

In the present discussion interest is focussed on the groups which might be important in supplying material for the starch industry, namely, sorgos and grain sorghums. The former have a disadvantage in that they grow tall and are therefore more difficult to harvest. But the recent universal acceptance of suitable new grain sorghum varieties and the use of the combine harvester-thresher will undoubtedly have a marked effect upon the agriculture of sorghum-producing areas. According to the Crop Reporting Board (1945) the acreage harvested for grain has increased from five million acres for the 1934–43 average to over six million for 1945. The average production of grain for the 1934–43 period was 70 million bushels, as compared with 96 million for 1945. Extensive work is being done at the Kansas, Nebraska, Oklahoma, and Texas Agricultural Experiment Stations by the plant breeders in an attempt to develop acceptable combine types. In view of the trend towards an increased production of sorghums and a greater demand for them by industry, the present work is concerned with collecting more information about sorghums and their starches.

Chemical Analyses of Sorghum Grains

To assist in the evaluation of the industrial potentialities of the many varieties of sorghums it is necessary to have proximate chemical analyses made on the grains. Some results on the standard varieties may be found in the literature, as reported by Baird (1910), Francis and Smith (1916), Edwards and Curtis (1943), MacMasters and Hilbert (1944), and Barham, *et al.* (1946), but due to the greatly expanded programs of the sorghum plant breeders in the past few years many new types have appeared. In all, 101 samples were analyzed, of which 14 were forage types and 87 grain sorghums. The method used for protein was the official A.O.A.C. (1940) Kjeldahl-Gunning method. The crude fat determinations were made by extracting the sorghum samples (ground to approximately 20-mesh) with Skellysolve "F-95" for six hours using a Butt extraction tube. The starch was determined by a polarimetric method used by Earle and Milner (1944) on corn samples.

The results of analyses for many of the newer varieties are summarized in Tables I and II. The results in Table I are arranged according to the four states from which samples were received. Table II contains more detailed information concerning the individual varieties examined. All of these grains were grown at the individual experiment stations, and for the most part represent different stages in the cross-breeding program. On an average basis, the values for the grain sorghums were somewhat higher in protein and starch and slightly

lower in oil than the forage sorghums. Since the grains were not all produced in the same year nor in the same locality, the data are insufficient to characterize the grains according to variety. The majority of the grain sorghums from the four states had approximately the same proportions of protein, crude fat, and starch, a fact which is important in considering their general industrial utilization. Some exceptional values were obtained with a Texas sample which analyzed 7.7% protein, 2.5% crude fat, and 81.8% starch. A low crude fat content of 2.5% was obtained with one Nebraska sample, whereas

TABLE I

CHEMICAL ANALYSES OF SORGHUM GRAINS
(Moisture-free basis)

State	No. of samples	Sample type	Average protein[1]	Range in protein	Average crude fat	Range in crude fat	Average starch	Range in starch
			%	%	%	%	%	%
Kansas	6	Forage	12.8	12.0–13.4	4.1	3.5–4.4	69.9	61.3–73.5
	3	Nonwaxy	12.7	12.0–13.1	3.9	3.5–4.4	70.6	68.0–72.4
	3	Waxy	12.9	12.1–13.4	4.3	4.2–4.4	69.2	61.3–73.5
	22	Grain	13.6	11.9–15.4	3.7	2.9–4.1	71.7	68.1–74.8
	19	Nonwaxy	13.7	12.1–15.4	3.7	2.9–4.1	71.4	68.1–74.8
	3	Waxy	13.0	11.9–13.5	3.5	3.3–3.6	73.6	72.2–74.4
	28	Forage and grain, all samples	13.5	11.9–15.4	3.8	2.9–4.4	71.3	61.3–74.8
Nebraska	8	Forage	10.9	9.1–13.6	3.9	3.2–4.4	68.8	48.0–78.5
	7	Nonwaxy	11.0	9.1–13.6	3.9	3.2–4.4	68.9	48.0–78.5
	1	Waxy	10.7	—	4.3	—	67.6	—
	39	Grain	12.0	8.7–15.2	3.5	2.5–4.5	71.9	66.6–77.7
	35	Nonwaxy	11.9	8.7–15.2	3.4	2.5–4.3	72.1	66.6–77.7
	4	Waxy	13.4	12.0–14.7	4.0	3.4–4.5	70.2	67.7–73.4
	47	Forage and grain, all samples	11.8	8.7–15.2	3.6	2.5–4.5	71.4	48.0–78.5
Oklahoma	0	Forage	—	—	—	—	—	—
	5	Grain	13.1	11.9–15.6	3.5	3.0–4.1	69.3	62.8–74.3
	2	Nonwaxy	14.4	13.2–15.6	3.4	3.0–3.8	66.5	62.8–70.3
	3	Waxy	12.3	11.9–12.7	3.5	3.2–4.1	71.1	67.3–74.3
	5	Forage and grain, all samples	13.1	11.9–15.6	3.5	3.0–4.1	69.3	62.8–74.3
Texas	0	Forage	—	—	—	—	—	—
	21	Grain	11.1	7.7–14.3	3.3	2.5–4.2	73.9	67.8–81.8
	7	Nonwaxy	9.8	7.7–11.4	3.5	2.5–4.2	75.1	67.8–81.8
	14	Waxy	11.8	8.9–14.3	3.2	2.5–3.8	73.3	71.1–77.0
	21	Forage and grain, all samples	11.1	7.7–14.3	3.3	2.5–4.2	73.9	67.8–81.8

[1] Protein calculated as N × 6.25.

4.3% was obtained with another one. The unusually low starch content of 48.0%, which was undoubtedly due to the glumes adhering to the kernels, was found in still another Nebraska variety.

Waxy starch, which contains approximately 100% of the branched chain starch fraction (amylopectin) and is most easily recognized by the reddish-brown color it stains with iodine, has been found in certain varieties of sorghum. The nonwaxy starch, which stains blue with iodine due to presence of the linear starch fraction (amylose), occurs in

the majority of sorghum varieties. In averaging the analytical results for the 87 samples of grain sorghums there appeared to be little difference in the proportions of protein, crude fat, and starch in samples which contained waxy and nonwaxy starches. The work of MacMasters and Hilbert (1944), however, indicated that there is a characteristic difference in the composition of waxy and nonwaxy types of sorghum, but their reported results were based on a limited number of samples.

Amylose Content of Sorghum Starches

Circumstances during the war created a demand for the waxy starches, principally as a replacement for tapioca starch, as reported by Hixon and Sprague (1942). The agronomists succeeded in developing

TABLE II

CHEMICAL ANALYSES OF SORGHUM GRAINS

Sample	Crop year	Moisture	(Moisture-free basis)		
			Protein	Crude fat	Starch
		%	%	%	%
Kansas:					
Forage, nonwaxy					
Atlas, C.I. 899	1944	9.7	12.0	4.4	72.4
Early Sumac, C.I. 1119	1944	10.7	13.0	3.5	68.0
Kansas Orange, C.I. 107	1944	10.4	13.1	4.0	71.6
Forage, waxy					
Waxy Atlas, H.C. 42-20	1944	9.6	12.1	4.3	73.5
Leoti Red, C.I. 1120	1944	10.6	13.4	4.4	61.3
Leoti x Atlas, H.C. 42-27	1944	9.6	13.2	4.2	72.8
Grain, nonwaxy					
Pink Kafir, C.I. 432	1944	9.7	12.6	3.7	71.0
Western Blackhull, C.I. 906	1944	9.8	13.6	3.7	71.9
Club Kafir, C.I. 901	1944	9.9	13.5	2.9	72.4
Finney Milo, C.I. 1089	1944	10.6	13.9	3.7	72.0
Feterita, C.I. 182-1	1944	9.7	15.4	3.5	70.2
Westland, G.C. 296	1944	9.6	13.4	3.8	68.9
Martin, S.A. 5330	1944	9.2	14.3	3.6	68.3
Early Kalo, C.I. 1009	1944	9.2	13.6	3.2	71.1
Midland, H.C. 617	1944	9.3	13.2	3.5	71.8
Wonder Club	1944	9.8	15.1	3.7	68.1
Dwf. Feterita x Dwf. Freed	1944	10.5	14.4	3.9	69.7
White Kalo Sel., 44-2112	1944	11.7	13.3	3.6	72.8
White Kalo Sel., 44-2117	1944	11.9	12.1	3.8	74.8
White Kalo Sel., 44-2119	1944	12.0	13.4	4.0	73.8
Leoti x (Club x Custer), 44-2115	1944	11.9	13.2	4.1	73.1
Waxy Hybrid, 44-2005	1944	12.1	13.2	3.8	73.4
Waxy Hybrid, 44-2021	1944	12.3	14.5	4.1	70.3
Waxy Hybrid, 44-2047	1944	12.2	13.7	3.6	70.9
Waxy Hybrid, 44-2049	1944	12.3	13.8	4.1	72.1
Grain, waxy					
Cody, H.C. 39-142	1944	10.2	11.9	3.3	74.4
Waxy Hybrid, 44-2020	1944	12.3	14.0	3.6	74.2
Waxy Hybrid, 44-2063	1944	12.0	13.5	3.6	72.2

TABLE II—(*Continued*)

Sample	Crop year	Moisture	(Moisture-free basis)		
			Protein	Crude fat	Starch
		%	%	%	%
Nebraska:					
Forage, nonwaxy					
Fremont, Nebr. 32	1944	9.0	12.9	4.0	67.0
Black Amber, F.D. 7038, Nebr. 19	1944	8.3	13.6	4.4	48.0
Kansas Orange, Nebr. 18	1944	9.7	9.6	4.2	71.4
Rox Orange, Nebr. 24	1944	9.9	10.0	3.8	71.3
S.A. 5023-19-4-3-1, N 176	1944	9.8	9.1	3.3	78.5
Norkan, C.I. 1116, Nebr. 38	1944	9.5	9.4	4.0	76.2
S.A. 5023-19-4-31, N 176	1945	9.0	12.1	3.2	70.4
Forage, waxy					
Leoti, C.I. 1120, Nebr. 26	1944	9.2	10.7	4.3	67.6
Grain, nonwaxy					
Caprock, Nebr. 58	1944	9.3	13.2	3.4	71.2
Western Blackhull, Nebr. 14	1944	9.4	9.7	3.3	75.7
Western Blackhull x Day, Nebr. 63	1944	9.9	12.1	3.5	73.3
Western Blackhull x Day, Nebr. 44	1944	9.3	9.6	4.1	72.9
Western Blackhull x Day, Nebr. 45	1944	9.5	13.4	3.6	72.7
Coes, Nebr. 28	1944	9.5	11.6	3.9	72.3
Club x Day No. 16, N 212	1944	9.5	9.3	3.1	74.8
Martin, Nebr. 59	1944	9.2	14.3	3.6	70.3
White Darso (Ks. 33-378), Nebr. 31	1944	9.6	11.2	4.3	69.5
Bonita, Nebr. 50	1943	9.4	13.4	3.0	71.3
Yellow Darso (813 x 615–36 x 12), N 134	1944	9.5	11.2	2.9	75.5
Chiltex, C.I. 874, Nebr. 27	1943	9.0	15.1	2.9	71.3
Club, C.I. 901, Nebr. 15	1944	9.3	12.6	2.8	75.0
Plainsman, Nebr. 57	1944	9.7	13.2	3.2	72.4
Highland, Nebr. 30	1944	9.5	11.4	4.0	72.1
Wd (871 x 792) x 71-3-4, N 194	1943	9.1	12.8	3.7	72.1
Alliance, Nebr. 33	1944	9.4	12.9	3.7	71.3
Sedan Red Kafir, N 25	1944	9.3	10.5	3.6	73.5
S.A. 7103-3-5-5, N 70	1944	9.0	10.1	3.4	74.9
S.A. 7103-8-6, N 71	1944	9.3	8.7	3.1	77.6
S.A. 7078-7-2-7-6, N 208	1044	9.1	10.4	3.3	76.6
S.A. 7103-3-6, N 69	1944	9.7	9.0	3.5	77.7
S.A. 7003-3-9-6, N 207	1944	8.8	10.7	3.4	71.6
S.A. 7097, N 68	1944	9.3	9.8	3.7	75.6
S.A. 5149-4-1, N 202	1944	9.1	11.8	2.5	72.8
S.A. 5252-1, N 205	1944	8.9	15.2	4.3	67.6
S.A. 5146-1, N 180	1944	9.0	11.1	3.1	71.5
S.A. 7009, N 170	1944	9.5	12.9	3.6	70.4
Schrock, Nebr. No. 27	1945	9.1	12.9	3.3	67.0
Yellow Darso, Nebr. N 134	1945	9.6	12.1	2.8	71.0
White Darso, Nebr. 31	1945	9.3	12.4	4.0	67.0
S.A. 7103-3-6, Nebr. 69	1945	8.4	11.0	3.5	69.2
S.A. 7103-8-6, Nebr. 71	1945	10.3	12.3	3.1	70.6
Martin, Nebr. 59	1945	8.7	14.8	3.6	66.6
Midland, Nebr. 47	1945	9.2	13.0	3.4	71.3
Grain, waxy					
Cody H.C. 39-140, N 193	1944	9.3	12.0	3.4	73.4
Waxy Kafir, S.A. 7113, Nebr. 48	1944	9.4	13.0	4.4	71.3
Waxy Milo, S.A. 5053, Nebr. 49	1943	9.0	14.7	3.8	67.7
Waxy Sooner, S.A. 5227-5, N 195	1943	8.9	14.0	4.5	68.6

TABLE II—(*Continued*)

Sample	Crop year	Moisture	(Moisture-free basis)		
			Protein	Crude fat	Starch
		%	%	%	%
Oklahoma:					
Grain, nonwaxy					
Shallu, C.I. 185	1945	10.2	15.6	3.8	62.8
Bonar (Durra) x Dwf. Brown Kaferita	1945	10.4	13.2	3.0	70.3
Grain, waxy					
Cody (Perkins)	1945	11.9	12.3	3.4	74.3
White Schrock	1945	11.8	12.0	4.1	67.3
71 x Leoti	1945	12.2	12.7	3.2	71.8
Texas:					
Grain, nonwaxy					
Waxy Club x Bonita, S.A. 5590-4	1945	9.0	7.7	2.5	81.8
Bonar Durra x Texas Dbl. Dwf., S.A. 5345-8-1-1	1945	9.1	9.7	3.6	76.2
Bonar Durra x Texas Dbl. Dwf., S.A. 5345-8-1-3	1945	9.3	9.5	2.9	75.6
Dbl. Dwf. White Sooner, S.A. 5155-5	1945	9.5	11.4	3.5	74.5
Dbl. Dwf. Yellow Sooner, S.A. 5155-36	1945	10.2	1.9	3.5	76.1
Shallu, S.A. 5375	1945	10.3	10.0	4.2	73.9
Dbl. Dwf. Shallu, S.A. 5523-18-2	1945	9.1	10.6	4.2	67.8
Grain, waxy					
Waxy Kafir, S.A. 5178	1945	9.0	14.0	3.4	71.3
Waxy Whiteseed, S.A. 5107-5-2-1	1945	9.3	11.5	3.8	72.8
Waxy Club x Waxy Whiteseed, S.A. 5580-2	1945	9.8	12.7	3.1	71.2
Waxy Club x Waxy Whiteseed, S.A. 5581-11	1945	9.6	11.2	3.4	77.0
Waxy Club x Waxy Whiteseed, S.A. 5581-27	1945	9.5	11.3	3.6	72.3
Waxy Club x Waxy Whiteseed, S.A. 5581-28	1945	9.4	12.0	2.7	75.3
Waxy Club x Bonita, S.A. 5590-50-1	1945	9.4	10.5	2.9	74.8
Waxy Club x Bonita, S.A. 5590-43	1945	8.3	11.5	2.5	73.7
Waxy Club x Bonita, S.A. 5590-50-2	1945	9.4	9.7	2.9	74.4
Dwf. Blackhull Waxy Kafir, S.A. 5036-1-3-4-2-2	1945	9.3	14.3	3.5	71.9
Dbl. Dwf. Waxy Blackhull, S.A. 5547	1945	9.1	12.9	3.3	74.1
Dwf. Yellow Waxy Milo, S.A. 7080	1945	9.0	13.0	3.2	71.1
Dwf. Yellow Waxy Milo, S.A. 5404	1945	9.5	8.9	3.5	72.7
Dalhart x Dwf. Yel. Waxy Milo, S.A. 5502-3-3	1945	8.8	11.5	3.7	73.3

suitable varieties of sorghum and other cereals which contained starches with practically 100% amylopectin characteristics. There are many indications that these starches will continue in importance in the years to come. At the same time, a starch with a very high proportion of amylose might also have industrial uses, possibly in the production of fibers, as indicated by Whistler and Hilbert (1944). For that reason, a search was made among the sorghum samples studied here for high-amylose starches.

Three methods are generally available for the estimation of the relative amounts of amylose and amylopectin in starches. (1) The starch may be dispersed in water by autoclaving and then saturated

with a polar organic substance, such as *n*-butanol, which causes the amylose to precipitate while the amylopectin remains in solution; this is Schoch's (1942) method. (2) The starch may be dissolved in potassium hydroxide, then neutralized and titrated with iodine solution by means of the electrometric method devised by Bates, French, and Rundle (1943). (3) The starch may be dissolved in sodium hydroxide, neutralized, and then diluted to give a final concentration of 0.002% starch. Iodine solution is added and light transmittance measurements are then made with a spectrophotometer at a wave length of 610 mμ, according to the procedure of Kerr and Trubell (1943). McCready and Hassid (1943) have proposed a similar method. All of these methods require that the starch be isolated from the plant material and be essentially free from noncarbohydrate matter.

In many instances, however, it is advantageous to be able to estimate the relative amounts of amylose and amylopectin without first isolating the starch. Such a rapid method has been developed by Kerr [3] and it consists principally in dispersing 2 g of the ground grain in calcium chloride solution (sp. gr. 1.30 at 20°C, pH of 2.5) and measuring the optical rotation of this solution in order to calculate the amount of starch present. An aliquot of this calcium chloride solution sufficient to contain 0.04 g of starch is then diluted with water so that a final starch concentration of 0.002% is obtained. Iodine solution is then added and the light transmittance is measured at 610 mμ. A standard curve, obtained by plotting the logarithm of the percentage transmittance against the percentage of amylose, was prepared by using an amylose and amylopectin which had been fractionated from a representative sample of sorghum starch.

When this method was applied to the sorghum samples containing nonwaxy starches, it was found that the percentage of amylose ranged from 27 to 45%. However, by first separating the starch from the grain, defatting it with methanol, and then titrating with iodine by the electrometric method of Bates, French, and Rundle (1943), the amylose contents ranged only from 25 to 31%. It is apparent then that the rapid method does not give absolute values for amylose content. Nevertheless, it is still useful in obtaining a relative evaluation of a large number of grain samples.[4] For example, most of the waxy samples analyzed between 0 and 5% amylose. On the other hand, a pea starch, which was known to contain a high proportion of amylose, gave a value of 90% by this method. It is, therefore, concluded that

[3] This method was obtained from Dr. R. W. Kerr of the Corn Products Refining Company in a private communication.

[4] A method developed by Nielsen and Gleason (1945) for the determination of starch by measuring the light transmission of starch-iodine solutions might also be useful in obtaining relative values of amylose content.

the nonwaxy sorghum samples studied here contained the normal amount of amylose, namely, from 25 to 30%, and that none had an exceptionally high percentage of amylose.

Properties of Sorghum Starches

The sorghum grains were processed for starch by using a laboratory method proposed by MacMasters and Hilbert (1944). The grain was steeped in 0.3% sulfur dioxide solution for 48 hours at 45°C. A 100-mesh metal screen sieve was used in place of the No. 17 standard bolting silk to separate the starch from the germ, bran, and larger fragments.

In general, the waxy starches did not separate so easily from the protein layer as the nonwaxy starches. It was also observed that the starch from grains containing water-soluble pigments was usually colored.

The pasting characteristics of the sorghum starches were examined by means of the Brabender Amylograph as described by Anker and Geddes (1944) and Brown and Harrel (1944), Scott and Stormer viscosities, and light transmittance studies. Typical amylograph curves for sorghum and corn starches, both waxy and nonwaxy, were made, employing 30 g of starch and 450 ml of distilled water. The viscosities of several sorghum and corn starches were determined by using the Stormer and Scott viscosimeters. Two-hundred ml of a 2% starch suspension was heated to boiling over an open flame, and then kept for exactly 10 minutes in a bath of boiling water. In both methods the viscosimeter cup was maintained at 90°C. The 100-g weight was used with the Stormer viscosimeter, and the time in seconds was recorded for one complete revolution of the dial pointer. In the case of the Scott viscosimeter the time in seconds required for 50 ml of the paste to flow through the orifice of the cup was recorded.

The transition point, which is defined as the temperature at which the first perceptible increase in viscosity takes place (Cook and Axtmayer, 1937), occurred in the amylograph curves at about 30 minutes for waxy and 40 minutes for nonwaxy starches, i.e., at a lower temperature for the waxy than for the nonwaxy (Figure 1). It is also apparent that the maxima of the curves for the waxy types are much larger than those for the nonwaxy. Microscopic examination showed that most of the nonwaxy starch granules had disintegrated completely after the peak in the curve had been reached. However, in the case of the waxy starches there were a few small, blue-staining granules (nonwaxy) which appeared to be unaffected during the pasting process.

The viscosity results, along with the maxima obtained with the amylograph, are summarized in Table III. In general, the viscosities

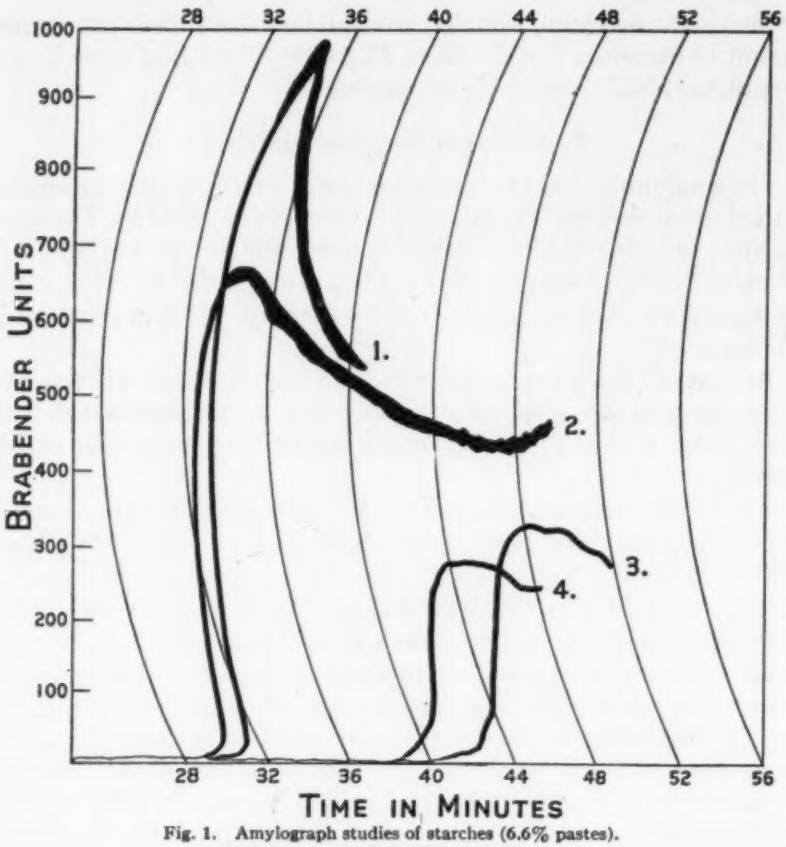


Fig. 1. Amylograph studies of starches (6.6% pastes).
1. Waxy sorghum (Cody H.C. 39–142)
2. Waxy maize
3. Nonwaxy sorghum (Atlas C.I. 899)
4. Nonwaxy corn

TABLE III

VISCOSITIES OF STARCHES

Sample	Stormer[1]	Scott	Brabender maxima
	Seconds	*Seconds*	
Corn starches			
Corn (commercial) (nonwaxy)	7	13	295
Waxy maize (commercial) (waxy)	9–10	17	680
Sorghum starches (nonwaxy)			
Western Blackhull, C.I. 906	8	14	400
Feterita, C.I. 182-1	8	14	365
Martin, S.A. 5330	8	13	400
Pink Kafir, C.I. 432	8	14	425
Atlas, C.I. 899	8	14	340
Sorghum starches (waxy)			
Leoti Red, C.I. 1120	15–12	37	900
Leoti x Atlas, H.C. 42-27	12–10	36	800
Cody, H.C. 39-142	16–12	58	990

[1] For the nonwaxy starches the Stormer values did not change with time. For the waxy starches, however, the time is recorded after the first and eleventh minute.

of the waxy starches were higher than those of the nonwaxy. The Stormer viscosities showed only small differences between corn and sorghum starches, either waxy or nonwaxy; the Scott viscosities differentiated more between the waxy corn and waxy sorghum; whereas the Brabender results indicated differences in both the waxy and nonwaxy types of corn and sorghum. The variations in the

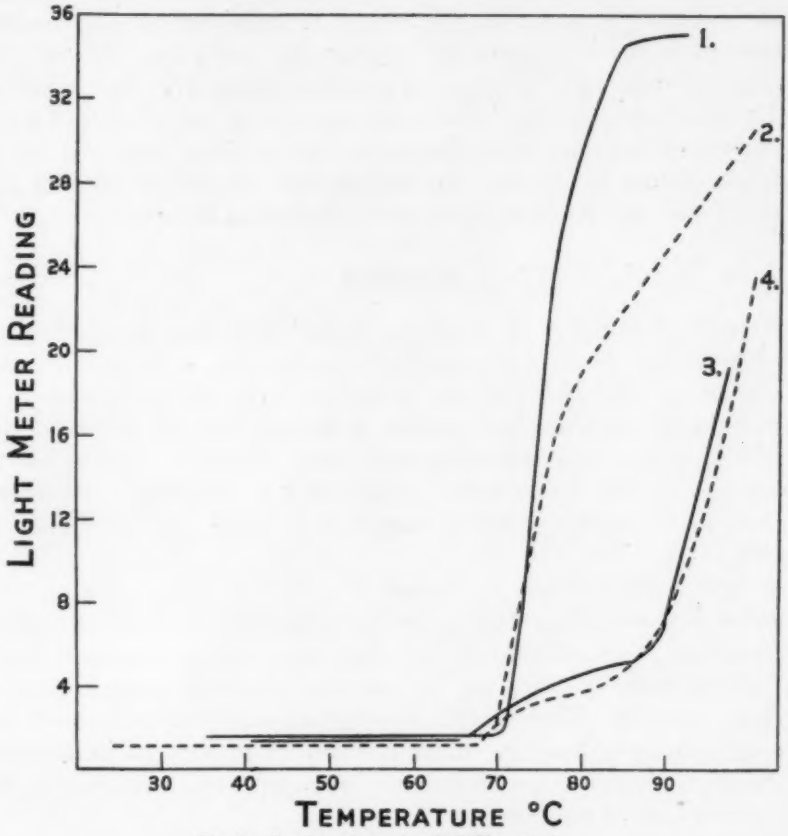


Fig. 2. Pasting of starches (0.51% pastes).
1. Waxy sorghum (Cody H.C. 39–142)
2. Waxy maize
3. Nonwaxy sorghum (Pink Kafir C.I. 432)
4. Nonwaxy corn

Brabender maxima for any one series of starches may be due to differences either in the grain variety or in the effects of processing. Somewhat similar viscosity results have been published by Barham and co-workers (1946).

The method of Morgan (1940) was used in studying the pasting properties of several samples of starch. This method is based on the change in light transmission of starch slurries during heat gelatiniza-

tion. Light from a 100-watt projection bulb was passed through a collimating lens on through the tube in which the paste was formed, and then onto the photoelectric cell. The heating was performed at the rate of about 2.5°C per minute. A Weston Illumination Meter, Model 603, was used to measure the intensity of the light. The values for the light transmitted, in arbitrary units, were plotted against the temperature. Representative results for corn and sorghum starches, given in Figure 2, point to the fact that there are no outstanding differences in the waxy starches of sorghum and corn; likewise, the nonwaxy starches of the same two cereal grains give quite similar light transmission curves. Photomicrographs of ungelatinized waxy and nonwaxy starch granules from corn and sorghum gave little or no evidence of any differences and contributed to the belief that the sorghum and corn starches have many properties in common.

Summary

Numerous samples of sorghum grains from Kansas, Nebraska, Oklahoma, and Texas were analyzed for protein, starch, and crude fat contents. The fact that the majority of the grain sorghums had approximately the same proportions of protein, starch, and crude fat may be important in considering their use in industry. In the line of exceptional varieties, one sample analyzed 7.7% protein, 2.5% crude fat, and 81.8% starch; another sample had a high crude fat content of 4.3%.

A rapid method involving the use of a saccharimeter and a spectrophotometer was employed for the estimation of the amylose contents of the sorghum starches. With the whole grain the results were high, but still of value in detecting any unusual properties with regard to amylose content. The nonwaxy sorghum samples contained the normal amount of amylose, namely, from 25 to 30% as determined by the electrometric iodine titration. No sample had an exceptionally high percentage of amylose.

Pasting characteristics of several waxy and nonwaxy sorghum starches, determined by means of the Brabender Amylograph, Scott and Stormer viscosities, and light transmission studies, showed a close similarity between corn and sorghum.

Acknowledgment

The authors wish to express appreciation to the Midwest Research Institute and to the Corn Products Refining Company for permitting the release of the data contained in this discussion. They also wish to thank the respective agricultural experiment stations for their complete cooperation in this work. Thanks are also due Dr. Ralph Kerr of Corn Products Refining Company for his suggestions concerning the determination of amylose in the whole grains.

Literature Cited

Anker, C. A., and Geddes, W. F.
1944 Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21**: 335–360.
Association of Official Agricultural Chemists
1940 Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 5th ed., p. 26.
Baird, A. O.
1910 The chemistry of the kafir corn kernel. Okla. Agr. Expt. Sta. Bull. 89.
Barham, H. N., Wagoner, J. A., Campbell, C. L., and Harclerode, E. H.
1946 The chemical composition of some sorghum grains and the properties of their starches. Kans. State Coll. Tech. Bull. 61.
Bates, F. L., French, D., and Rundle, R. E.
1943 Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65**: 142–148.
Brown, R. O., and Harrel, C. G.
1944 The use of the amylograph in the cereal laboratory. Cereal Chem. **21**: 360–369.
Cook, D. H., and Axtmayer, J. H.
1937 Determining the gelatinization temperature of starches. Ind. Eng. Chem. (Anal. Ed.) **9**: 226–228.
Crop Reporting Board
1945 Bureau of Agricultural Economics, U. S. Dept. Agr., September 10.
Earle, F. R., and Milner, R. T.
1944 Improvements in the determination of starch in corn and wheat. Cereal Chem. **21**: 567–575.
Edwards, W. M., and Curtis, J. J.
1943 Grain sorghums, their products and uses. U. S. Dept. Agr., Bureau of Agr. and Ind. Chem. ACE-193, NM-229.
Francis, C. K., and Smith, O. C.
1916 The starches of the grain sorghums. Okla. Expt. Sta. Bull. No. 110.
Hixon, R. M., and Sprague, G. F.
1942 Waxy starch of maize and other cereals. Ind. Eng. Chem. **34**: 959–962.
Kerr, R. W., and Trubell, O. R.
1943 Spectrophotometric analyses of starches. Paper Trade Journal, Oct. 7.
MacMasters, M. M., and Hilbert, G. E.
1944 Glutinous corn and sorghum starches. Ind. Eng. Chem. **36**: 958–963.
Martin, J. H.
1937 Sorghum improvement. U. S. Dept. Agr. Yearbook Separate No. 1575, 523–560.
McCready, R. M., and Hassid, W. Z.
1943 The separation and quantitative estimation of amylose and amylopectin in potato starch. J. Am. Chem. Soc. **65**: 1154–1157.
Morgan, W. L.
1940 Pasting and identification of starches. Ind. Eng. Chem. (Anal. Ed.) **12**: 313–317.
Nielsen, J. P., and Gleason, P. C.
1945 Rapid determination of starch. Ind. Eng. Chem. (Anal. Ed.) **17**: 131–134.
Schoch, T. J.
1942 Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. **64**: 2957–2961.
Slotter, R. L., and Langford, C. T.
1944 Wheat starch manufacture. Ind. Eng. Chem. **36**: 404–408.
Whistler, R. L., and Hilbert, G. E.
1944 Mechanical properties of films from amylose, amylopectin, and whole starch acetates. Ind. Eng. Chem. **36**: 796–798.

COMPOSITION OF THE COMPONENT PARTS OF THE CORN KERNEL

F. R. EARLE, J. J. CURTIS, and J. E. HUBBARD

Northern Regional Research Laboratory,[1] Peoria, Illinois

(Received for publication May 10, 1946; presented at the Annual Meeting, May 1946)

The breeding of corn for commercial production has been guided almost entirely by agronomic factors such as yield, general appearance of the plant and grain, and resistance to disease and lodging. It is generally agreed that it would be desirable to have a high-protein corn for feeding and a high-oil corn for milling, but these factors have been given little consideration by the corn producers. Unless the milling industry can offer special inducements, production of high-oil corn is unlikely unless the agronomic characteristics are at least as favorable as those of other available varieties. There are significant differences in the oil contents of the hybrid corn varieties which are widely grown at the present time. A study of some of these varieties shows the possibilities of advantage in processing high-oil corn.

It should be emphasized that the data in this paper indicate that better hybrids can be developed for processing but are not to be considered as recommendation or condemnation of specific varieties.

If the high-oil corn is to be of special value to industry, it is essential that the increase in oil be largely in the germ. In order to show the location of the oil in current varieties of corn, 11 samples have been carefully separated by hand into four portions consisting of endosperm, germ, bran, and tip-cap. The samples were selected to include one yellow and one white hybrid variety of high-oil content and one each of low-oil content. Three other yellow hybrid varieties of intermediate oil content were included, one of them being represented by two samples grown at different stations in 1945 and by one sample grown at one of the same stations in 1942. A flint and a flour corn were also included to see if such diverse types would have unusual proportions.

Methods

The method of separation was similar to that of Hopkins, Smith, and East (1903) except that cold water was used to soak the corn to loosen the bran and the time of soaking was kept to the minimum necessary to permit the hand separation, usually between 5 and 30 minutes. In order to get an indication of the uniformity of the samples, each sample was divided into four portions of about 75 g and each

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

portion separated into endosperm, germ, bran, and tip-cap fractions. After air-drying, the fractions were examined for completeness of separation and weighed. The physical proportions determined on the four replications were in such good agreement that the replicates were combined and only the average result is reported.

In preparing the samples for analysis, the endosperm was ground in a hammer mill, the bran and tip-cap in an intermediate model Wiley mill, and the germ was coarsely ground in a small attrition mill. Since the samples were air-dried before grinding it is considered that there would be no great change in moisture during grinding. The tip-cap fraction was so small that it was necessary to use composites for analysis. One composite represented six of the yellow double-cross hybrids and the other represented the flint corn, the flour corn, and the two white single-cross hybrid strains. These two composites had such similar analyses that the average of the two was used in connection with each of the 10 samples of grain. The tip-cap from the remaining sample was obtained later and the amount was so small that only a partial analysis could be made.

Samples of the grain and the fractions resulting from the separation were analyzed for moisture, ash, nitrogen, oil, sugar, and starch. The methods of the Association of Official Agricultural Chemists (1940) were used to determine moisture, ash, nitrogen, and sugar. Oil was determined as in the A.O.A.C. method for oil in cottonseed except that the sample was unwrapped after about 4 hours, ground in a mortar, and then extracted overnight. Starch was determined by the Clendenning polarimetric method (1945) using uranyl acetate as the protein precipitant.

Discussion

All analyses are reported on the moisture-free basis.

The identity of the corn samples and the chemical composition are shown in Table I. The physical and chemical composition of the fractions is shown in Table II. In Table III, derived from Table II by calculation, is shown the amount of each determined constituent in each fraction expressed as the percentage of the whole kernels. Also in Table III is shown the amount of each constituent which occurs in each fraction expressed as the percentage of the total amount of that constituent in the whole kernels.

The composition of the whole corn kernel was calculated from the composition of its parts, as given in the first part of Table III, by totaling for each constituent the percentage found in the four fractions of each sample of whole kernels. This calculated composition has been included in Table I for comparison with the composition obtained

directly by analysis of the kernels. In general, the agreement is excellent, the principal disagreement being in the figures for starch in the flint and flour corns. Some of the differences in the ash and sugar results can be explained by the extraction of solubles from the whole grain during the soaking period. The amount of solubles was not measured for these samples, but in some later work it was found to be

TABLE I

COMPOSITION OF WHOLE CORN

Sample No.	Variety	Source	Crop year	Wt. per 1,000 kernels	Moisture-free basis					
						Ash	Protein (N × 6.25)	Oil	Sugar	Starch
				g		%	%	%	%	%
1	Iowa 939	Ames, Ia.	1942	305	Determined	1.52	8.2	3.9	1.77	74.0
					Calculated[1]	1.64	8.3	4.1	1.80	74.2
2	U. S. 13	Ames, Ia.	1942	284	Determined	1.47	8.1	4.3	1.79	73.7
					Calculated	1.45	7.8	4.2	1.56	74.0
3	U. S. 13	Ames, Ia.	1945	257	Determined	1.33	9.8	4.3	1.61	73.7
					Calculated	1.33	9.6	4.3	1.60	73.5
4	U. S. 13	Wooster, O.	1945	260	Determined	1.47	11.1	4.7	1.74	72.0
					Calculated	1.44	10.9	4.7	1.81	73.0
5	Illinois 201	Ames, Ia.	1942	299	Determined	1.58	8.4	4.7	2.22	73.1
					Calculated	1.67	8.3	5.0	1.71	73.6
6	U. S. 44	Ames, Ia.	1942	273	Determined	1.39	8.2	4.7	2.06	74.2
					Calculated	1.50	8.1	4.8	2.03	74.4
7	Illinois 200[2]	Illinois	1942	315	Determined	1.48	11.5	5.6	2.20	67.8
					Calculated	1.50	11.7	5.8	2.07	68.7
8	Ind. 33–16 × K64	Chillicothe, O.	1944	305	Determined	1.27	9.6	4.1	2.12	73.4
					Calculated	1.38	9.4	4.2	1.92	73.9
9	T18 × Tx4R3	Chillicothe, O.	1944	418	Determined	1.35	11.4	5.8	2.01	70.0
					Calculated	1.44	11.4	5.9	1.89	70.6
10	Gehu Flint	Bismarck, N. Dak.	1940	215	Determined	1.51	13.6	5.6	2.03	67.8
					Calculated	1.55	13.4	5.5	1.97	69.8
11	Mandan White Flour	Bismarck, N. Dak.	1940	225	Determined	1.49	13.2	5.6	2.14	66.8
					Calculated	1.68	13.3	5.7	2.07	69.1
	Average				Determined	1.44	10.3	4.8	1.97	71.5
	High				Determined	1.58	13.6	5.8	2.22	74.2
	Low				Determined	1.27	8.1	3.9	1.61	66.8

[1] Calculated from data in Tables II and III.
[2] Composite of seven samples.

not more than 0.25% of the total sample. Of this amount approximately one-fourth was ash and one-fourth protein, and the remainder was assumed to be carbohydrate. Unless there is a special interest in the sugar or protein content of the bran, it is believed that the errors due to the extraction of soluble materials are insignificant.

The results of this study of hybrid corn are in excellent agreement with the more extensive study made in 1903 by Hopkins, Smith, and

TABLE II

Composition of Corn Fractions
(Moisture-free Basis)

Sample No.	Variety	Proportion of whole grain	Ash	Protein (N × 6.25)	Oil	Sugar	Starch
		%	%	%	%	%	%
	Endosperm Portion						
1	Iowa 939	83.0	0.46	7.2	0.8	0.77	87.8
2	U. S. 13	82.7	.32	6.7	.7	.47	88.0
3	U. S. 13	83.5	.23	8.8	.8	.48	86.9
4	U. S. 13	82.7	.22	10.4	.9	.82	86.4
5	Illinois 201	82.1	.46	7.2	1.0	.48	87.8
6	U. S. 44	81.8	.27	6.9	.8	.70	88.9
7	Illinois 200[1]	80.3	.28	11.1	.9	.64	83.9
8	Ind 33–16 × K64	83.0	.32	8.6	.8	.64	87.2
9	T18 × Tx4R3	81.7	.23	10.8	1.1	.49	84.5
10	Gehu Flint	80.6	.24	12.8	.8	.73	84.7
11	Mandan White Flour	79.7	.33	12.8	.8	.81	84.7
	Average	81.9	.31	9.4	.8	.64	86.4
	High	83.5	.46	12.8	1.1	.82	88.9
	Low	79.7	.22	6.7	.7	.47	83.9
	Germ or Embryo Portion						
1	Iowa 939	10.5	11.31	18.9	31.1	10.36	9.0
2	U. S. 13	11.0	10.22	18.0	32.6	10.36	9.2
3	U. S. 13	10.2	10.50	19.0	34.9	11.46	5.1
4	U. S. 13	10.9	11.04	18.4	35.1	10.13	8.8
5	Illinois 201	11.9	10.31	17.3	34.0	10.80	8.1
6	U. S. 44	12.3	10.15	18.3	33.1	10.00	8.0
7	Illinois 200[1]	12.8	9.67	20.0	38.9	11.95	6.2
8	Ind 33–16 × K64	10.9	9.87	18.7	31.6	12.51	10.0
9	T18 × Tx4R3	13.1	9.38	18.2	37.9	11.51	10.0
10	Gehu Flint	13.5	9.50	20.2	35.3	10.00	8.0
11	Mandan White Flour	14.1	9.16	19.9	35.0	9.87	8.0
	Average	11.9	10.10	18.8	34.5	10.81	8.2
	High	14.1	11.31	20.2	38.9	12.51	10.0
	Low	10.2	9.16	17.3	31.1	9.87	5.1
	Bran Portion						
1	Iowa 939	5.7	1.01	3.8	1.2	.52	7.0
2	U. S. 13	5.5	.97	3.3	.7	.27	3.5
3	U. S. 13	5.2	.90	3.9	.9	.34	6.5
4	U. S. 13	5.4	.86	3.8	1.1	.36	8.2
5	Illinois 201	5.3	.97	3.8	1.2	.39	10.4
6	U. S. 44	5.1	.36	3.0	1.1	.29	7.7
7	Illinois 200[1]	6.2	.36	3.8	.7	.28	8.0
8	Ind 33–16 × K64	5.1	.36	2.9	.8	.19	7.0
9	T18 × Tx4R3	4.4	.29	3.6	1.1	.42	7.5
10	Gehu Flint	5.1	1.18	4.6	1.0	.32	7.0
11	Mandan White Flour	5.5	2.00	4.6	.9	.32	7.4
	Average	5.3	.84	3.7	1.0	.34	7.3
	High	6.2	2.00	4.6	1.2	.52	10.4
	Low	4.4	.29	2.9	.7	.19	3.5
	Tip Cap Portion						
3	U. S. 13	1.1	1.95	10.7	3.9	—	—
	Composite [2]	.8 (Av.)	1.59	9.1	3.8	1.61	5.3
	Composite [3]	.8 (Av.)	1.43	9.2	3.7	1.44	5.3

[1] Composite of seven samples.
[2] Composite of six yellow dent varieties.
[3] Composite of one flint, one flour, and two white dent varieties.

TABLE III

Distribution of the Chemical Constituents in Corn
(Data Derived from Tables I and II)

Sample No.	Variety	Proportion of whole grain existing as indicated constituent in specified fraction					Proportion of total of indicated constituent existing in specified fraction				
		Ash	Protein (N ×6.25)	Oil	Sugar	Starch	Ash	Protein (N ×6.25)	Oil	Sugar	Starch
		%	%	%	%	%	%	%	%	%	%
	Endosperm Portion										
1	Iowa 939	0.38	6.0	0.7	0.64	72.9	23.3	72.6	16.6	36.1	98.1
2	U. S. 13	.26	5.5	.6	.39	72.8	18.2	71.3	13.3	25.0	98.3
3	U. S. 13	.19	7.3	.6	.40	72.6	14.4	76.4	14.6	25.0	98.7
4	U. S. 13	.18	8.6	.8	.68	71.4	12.6	78.9	16.6	37.3	98.0
5	Illinois 201	.38	6.0	.9	.39	72.1	22.7	72.0	17.4	23.0	97.9
6	U. S. 44	.22	5.6	.6	.57	72.7	14.7	69.5	13.3	28.2	97.8
7	Illinois 200 [2]	.22	8.9	.7	.51	67.4	15.0	75.7	12.8	24.8	98.1
8	Ind 33–16 × K64	.27	7.2	.7	.53	72.4	19.4	75.8	16.2	27.7	97.9
9	T18 × Tx4R3	.19	8.8	.9	.40	69.0	13.0	77.1	15.1	21.2	97.8
10	Gehu Flint	.19	10.3	.6	.59	68.3	12.5	77.3	11.5	29.9	97.9
11	Mandan White Flour	.26	10.2	.7	.65	67.5	15.7	76.6	11.9	31.2	97.7
	Average	.25	7.7	.7	.52	70.8	16.5	74.8	14.5	28.2	98.0
	High	.38	10.3	.9	.68	72.9	23.3	78.9	17.4	37.3	98.7
	Low	.18	5.5	.6	.39	67.4	12.5	69.5	11.5	21.2	97.7
	Germ or Embryo Portion										
1	Iowa 939	1.19	2.0	3.3	1.09	0.9	72.4	23.9	81.0	61.5	1.3
2	U. S. 13	1.12	2.0	3.6	1.14	1.0	77.3	25.5	85.0	73.3	1.3
3	U. S. 13	1.07	1.9	3.6	1.17	.5	80.5	20.3	83.3	72.8	.7
4	U. S. 13	1.20	2.0	3.8	1.10	1.0	83.2	18.4	81.4	60.8	1.3
5	Illinois 201	1.23	2.1	4.0	1.28	1.0	73.6	24.9	80.9	75.1	1.3
6	U. S. 44	1.25	2.2	4.1	1.43	1.2	83.3	27.8	84.9	70.5	1.6
7	Illinois 200 [2]	1.24	2.6	5.0	1.53	.8	82.8	21.8	86.0	73.8	1.1
8	Ind 33–16 × K64	1.08	2.0	3.4	1.36	1.1	78.2	21.6	81.9	71.0	1.5
9	T18 × Tx4R3	1.23	2.4	5.0	1.46	1.2	85.2	20.9	83.6	77.2	1.7
10	Gehu Flint	1.28	2.7	4.8	1.35	1.1	82.9	20.4	87.1	68.7	1.5
11	Mandan White Flour	1.29	2.8	4.9	1.39	1.1	77.1	21.1	86.8	67.4	1.6
	Average	1.20	2.3	4.1	1.30	1.0	79.7	22.4	83.7	70.2	1.4
	High	1.29	2.8	5.0	1.53	1.2	85.2	27.8	87.1	77.2	1.7
	Low	1.07	1.9	3.3	1.09	.5	72.4	18.4	80.9	60.8	.7

[1] * = less than 0.05%.

[2] Composite of seven samples.

TABLE III—*Continued*

TABLE III—*Continued*

Sample No.	Variety	Proportion of whole grain existing as indicated constituent in specified fraction					Proportion of total of indicated constituent existing in specified fraction				
		Ash	Protein (N×6.25)	Oil	Sugar	Starch	Ash	Protein (N×6.25)	Oil	Sugar	Starch
		%	%	%	%	%	%	%	%	%	%
	Bran Portion										
1	Iowa 939	0.05	0.2	0.1	0.03	0.4	3.5	2.6	1.7	1.7	0.5
2	U. S. 13	.05	.2	*[1]	.02	.2	3.6	2.3	1.0	1.0	.3
3	U. S. 13	.05	.2	*	.02	.3	3.5	2.1	1.1	1.1	.5
4	U. S. 13	.05	.2	.1	.02	.4	3.2	1.8	1.3	1.1	.6
5	Illinois 201	.05	.2	.1	.02	.6	3.1	2.4	1.2	1.2	.7
6	U. S. 44	.02	.2	.1	.02	.4	1.2	1.8	1.2	.7	.5
7	Illinois 200 [2]	.02	.2	*	.02	.5	1.5	2.0	.8	.8	.7
8	Ind 33–16 × K64	.02	.2	*	.01	.4	1.3	1.6	1.0	.5	.5
9	T18 × Tx4R3	.01	.2	*	.02	.3	.9	1.4	.8	1.0	.4
10	Gehu Flint	.06	.2	*	.02	.4	3.9	1.7	.9	.8	.5
11	Mandan White Flour	.11	.2	.1	.02	.4	6.6	1.9	.9	.8	.6
	Average	.05	.2	.1	.02	.4	2.9	2.0	1.1	1.0	.5
	High	.11	.2	.1	.03	.6	6.6	2.6	1.7	1.7	.7
	Low	.01	.2	*	.01	.2	.9	1.4	.8	.5	.3
	Tip Cap Portion										
1	Iowa 939	0.01	0.1	*	0.01	*	0.7	0.9	0.8	0.7	0.1
2	U. S. 13	.01	.1	*	.01	*	.8	.9	.7	.8	.1
3	U. S. 13	.02	.1	*	.02	0.1	1.6	1.2	1.0	1.1	.1
4	U. S. 13	.02	.1	*	.02	.1	1.0	.8	.8	.8	.1
5	Illinois 201	.01	.1	*	.01	*	.7	.7	.5	.6	.1
6	U. S. 44	.01	.1	*	.01	*	.8	.9	.6	.6	.1
7	Illinois 200 [2]	.01	.1	*	.01	*	.7	.5	.4	.5	.1
8	Ind 33–16 × K64	.02	.1	*	.02	.1	1.1	1.0	.9	.8	.1
9	T18 × Tx4R3	.01	.1	*	.01	*	.8	.6	.5	.6	.1
10	Gehu Flint	.01	.1	*	.01	*	.8	.5	.6	.6	.1
11	Mandan White Flour	.01	.1	*	.01	*	.7	.4	.5	.5	.1
	Average	.01	.1	*	.01	*	.9	.8	.7	.7	.1
	High	.02	.1	*	.02	.1	1.6	1.2	1.0	1.1	.1
	Low	.01	.1	*	.01	*	.6	.5	.4	.5	.1

[1] * = less than 0.05%.

[2] Composite of seven samples.

East on their strains of corn selected for high and low protein and for high and low oil. This probably means only that hybridization has not changed the physical proportions of the kernel and that any conclusions to be drawn from the early data will apply equally well to present-day hybrids.

The utilization of the data of this study will depend entirely on the interest of the user. The primary purpose of this paper is to show the distribution of oil in the corn kernel and only relevant data will be discussed. For other purposes many interesting relationships might be found.

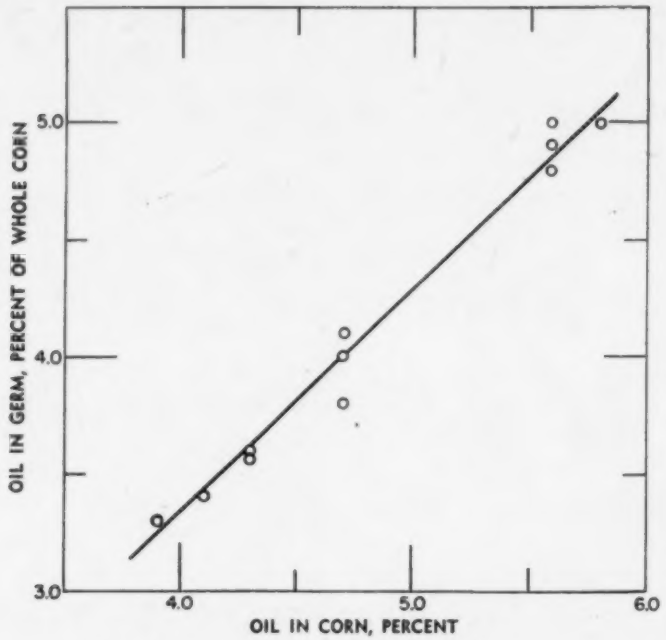


Fig. 1. Relation of oil in germ and oil in whole corn.

Figure 1 shows that proportion of the whole grain which occurs as oil in the germ plotted against the total oil in the sample. The correlation between the protein in the endosperm and that in the whole grain is equally good. Use of Figure 1 permits an estimate of the amount of oil which might possibly be recovered in processing a sample of corn of known total oil content. In illustration of this use, Table IV shows the possible recovery of oil from high-oil and low-oil corn. The calculations are based on 100 pounds of moisture-free corn and it is assumed that 100% of the germ will be separated free of other material and that the germ cake will contain 5% oil. These figures can be modified for any particular installation where the percentage of germ recovery and

TABLE IV
OIL YIELD FROM CORN
(Based on 100 pounds of moisture-free corn)

	Ill. 200	Iowa 939
	Pounds	*Pounds*
Weight of oil in kernels	5.6	3.9
Weight of germ	12.8	10.5
Weight of oil in germ	5.0	3.3
Weight of oil in germ cake (5% residual oil)	.4	.4
Weight of oil recovered	4.6	2.9

the amount of nongerm material in the germ cake are known. Incomplete recovery of germ would reduce the oil yield proportionately. The presence of nongerm materials and excess oil in the germ cake would reduce the yield by equal amounts in the processing of high- and low-oil corn but the percentage reduction would be greater for the low-oil corn.

Summary

Eleven samples of corn grain were separated by hand into fractions representing the endosperm, germ, bran, and tip-cap. The grains and the resulting fractions were analyzed for moisture, ash, nitrogen, oil, sugar, and starch. The endosperm contains approximately 16% of the total ash, 75% of the total protein, 15% of the oil, 28% of the sugar, and 98% of the starch. The germ contains approximately 80% of the total ash, 22% of the protein, 84% of the oil, and 70% of the sugar. The data show high correlation between the amount of oil in the grain and the amount in the germ.

Acknowledgment

Seed samples were supplied by the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Iowa, Illinois, and Ohio experiment stations.

Literature Cited

Association of Official Agricultural Chemists
1940 The official and tentative methods of analysis (5th ed.).
Clendenning, K. A.
1945 Polarimetric determination of starch in cereal products. IV. Critical studies of methods for the determination of starch in whole wheat, granular, and patent flours. Can. J. Research **B23**: 239–259.
Hopkins, C. G., Smith, L. H., and East, E. M.
1903 The structure of the corn kernel and the composition of its different parts. Ill. Agr. Expt. Sta. Bull. 87.

INFLUENCE OF INGREDIENTS ON THIAMINE RETENTION AND QUALITY IN BAKING POWDER BISCUITS

ALICE M. BRIANT and MARCIA R. HUTCHINS

New York State College of Home Economics and Agricultural Experiment Station, Cornell University, Ithaca, New York

(Received for publication June 14, 1946)

In self-rising flour biscuits, Barackman (1942) reported 85% of the thiamine was retained if the pH of the baked product was 7.1 or lower. He obtained slightly better retention of thiamine when fluid milk was substituted for water. Williams and Fieger (1944) reported that thiamine content of enriched-flour biscuits decreased with increase in the pH of the dough mixture.

Because little is known of the retention of thiamine in home-baked enriched-flour products, a study was made of the effect of certain ingredients on thiamine retention in baking powder biscuits made by a household method. The effect of the variables on the quality of the biscuits was also studied.

Materials and Methods

A basic formula (Table I) was used for the biscuits, and variations from this were made in liquid and in leavening agent (Table II). A standard household method of preparing the biscuit dough was used.

TABLE I

BASIC FORMULA FOR BISCUITS

Ingredient	Weight
Flour, pastry	330 g [1]
Salt	6.0 g
Fat, hydrogenated vegetable	75.0 g
Baking powder, sodium aluminum sulfate-phosphate	12.3 g
Fluid whole milk	240 ml

[1] From 3 to 6 g additional flour was used in kneading and rolling.

The dough was rolled to $\frac{3}{8}$-inch thickness, and the biscuits were cut two inches in diameter. They were spaced uniformly on a baking sheet and baked in a household type gas oven at 450°F for 12 minutes, or for 15 minutes when water was the liquid.

In Table II, the experiments are grouped according to the ingredients studied. For each day's baking, one batch for each variable in the group was prepared. In all, five batches for each variable were baked. A different bag of flour was used for each group of experiments, but all the flour was from the same mill lot.

Sampling methods were designed to reduce to a minimum the variation due to manipulation and to lack of homogeneity of the dough. The position of the biscuits relative to each other was maintained throughout the cutting, baking, and cooling processes. The different

TABLE II

VARIATIONS IN LIQUID AND LEAVENING MADE IN THE BASIC FORMULA

Group	Ingredients	Amount
I. Different liquids	Fresh whole milk	240 ml
	Evaporated milk	120 ml
	plus water (distilled)	120 ml
	Dry whole milk [1]	29 g
	plus water (distilled)	214 ml
	Water (distilled)	214 ml
II. Different types of baking powder	Sodium aluminum sulfate-phosphate baking powder (3 brands)	12.3 g [2]
	Phosphate baking powder	19.3 g [2]
	Tartrate baking powder	22.8 g [2]
III. Different amounts of baking powder	Sodium aluminum sulfate-phosphate baking powder	12.3 g [2]
	Sodium aluminum sulfate-phosphate baking powder	18.4 g
IV. Constant amount of lactic acid with varying amounts of baking soda [3]	Lactic acid	2.05 g [4]
	Sodium bicarbonate	1.64 g [5]
	Sodium aluminum sulfate-phosphate baking powder	10.28 g
	Lactic acid	2.05 g
	Sodium bicarbonate	2.50 g
	Sodium aluminum sulfate-phosphate baking powder	6.54 g
	Lactic acid	2.05 g
	Sodium bicarbonate	3.00 g
	Sodium aluminum sulfate-phosphate baking powder	4.52 g
	Lactic acid	2.05 g
	Sodium bicarbonate	3.50 g
	Sodium aluminum sulfate-phosphate baking powder	2.16 g
	Lactic acid	2.05 g
	Sodium bicarbonate	4.50 g
	Sodium aluminum sulfate-phosphate baking powder	0.00 g

[1] Whenever dry whole milk was used it was mixed with the dry ingredients.
[2] This amount is that recommended by the manufacturer for use with the amount of flour in the basic recipe.
[3] 29 g of dry whole milk plus 214 ml distilled water were used in these doughs. The amount of baking powder was varied to keep the total leavening as nearly constant as possible.
[4] This amount of lactic acid approximates that in 240 ml of full soured milk.
[5] This amount of soda is chemically equivalent to the lactic acid used.

positions were numbered according to the scheme in Figure 1. Four of the biscuits (Nos. 1, 5, 9, 10) were used for subjective judgment, four (Nos. 3, 4, 8, and 12) were used for objective measurements, and four (Nos. 2, 6, 7, 11) were used for thiamine, dry matter, and pH determinations.

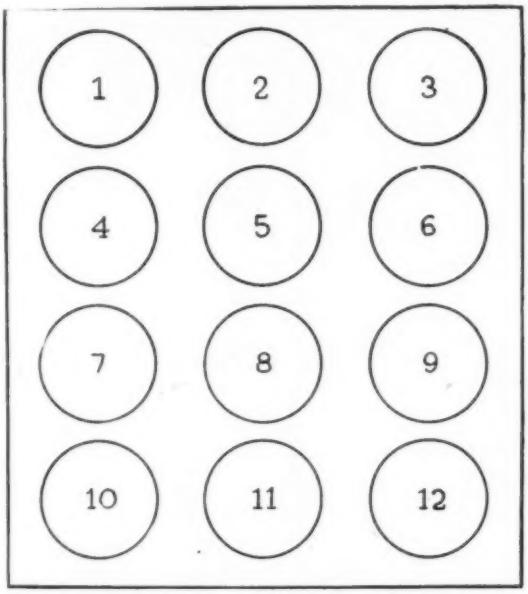


Fig. 1. The position of the biscuits relative to each other.

Chemical Determinations

Sampling Methods. The biscuits were sampled 15 minutes after they were taken from the oven. Two opposite quarters of biscuit number 2 and two of biscuit number 6 were ground together in a mortar until fairly fine and crust and crumb were intimately mixed. One sample of this mixture was taken for each of the determinations of thiamine, dry matter, and pH. The procedure was repeated for biscuits 7 and 11.

Thiamine, dry matter, and pH of the dough were also determined. Dough samples were taken and weighed immediately after the biscuits had been cut. One sample for each determination was taken from around positions 2 and 6 and a second from around positions 7 and 11. The dough samples were taken from the positions adjacent to the biscuits used for similar determinations because preliminary experiments had shown this lessened the chance of large differences in thiamine and pH values between the dough used for the determinations and that in the biscuits.

Thiamine in the flour and milk was also determined, and these values were used in calculating the amount of thiamine in the doughs.

Analytical Methods. Thiamine was determined by a thiochrome method, essentially that of Conner and Straub (1941). All samples were blended with the acid immediately after being weighed. Dry matter was determined by the method of the American Association of Cereal Chemists (1941). A Beckman pH meter was used to measure pH. The determinations were made on the undiluted dough and on a slurry of one part of ground biscuit to two parts distilled water (McKim and Moss, 1943). Preliminary experiments had shown the error of measurement on the undiluted dough to be small. Determinations of pH could not be made on the undiluted biscuit.

Subjective judgments were made by three experienced judges on the hot biscuits 10 minutes after being taken from the oven. The biscuits were scored for external and internal color, tenderness of crust and crumb, flakiness, cell size, moisture, and flavor. The maximum score was 100.

Objective measurements were made after the biscuits had cooled to room temperature. The height of the four biscuits set one on top of the other was measured in centimeters.

Compressibility of the crumb was measured using the Baker compressimeter [1] described by Platt and Powers (1940). A one-centimeter slice was cut from the bottom of the biscuit. The effect of the side crust was eliminated by cutting out a two-inch disc from the center of this slice and using this for the measurement of compressibility.

The breaking strength of the bottom crust was measured using an instrument similar in principle to the Bailey shortometer (1934) in which weight is added gradually to a blunt knife-edge pressing down on the piece to be broken. The addition of weight ceased abruptly when the crust was broken, and this weight was recorded as "breaking strength."

Results and Discussion

The results of the complete study are shown in Table III. Each figure is the average of the values obtained on five batches. The coefficients of variability were 5% or below for thiamine retention, measurement of height, and judges' scores, and were from 10 to 20% for the hydrogen ion concentration, thiamine content, compressibility, and breaking strength.

The thiamine content of the flour and its water absorption capacity varied with the different bags in spite of the fact that all four bags

[1] The authors are indebted to the Borden Company, Syracuse, New York, for the loan of the compressimeter.

TABLE III

THIAMINE RETENTION, pH, AND QUALITY OF BISCUITS
(Averages for 5 batches)

Group	Variable	Thiamine[1]				pH		Objective measurements			Palatability score[5]
		Dough		Biscuit		Dough	Biscuit	Compressibility[4]	Breaking strength	Height of four biscuits	
		Calculated[2]	Found	Found	Retention[3]						
		μg/g	μg/g	μg/g	%			mm	g	cm	
I. Different liquids	Fresh milk	3.5	3.3	3.1	89	7.0	6.8	1.69	365	15.6	92
	Evaporated milk	3.4	3.2	2.8	84	7.1	6.6	1.43	309	13.2	80
	Dry milk plus water	3.5	3.2	2.9	83	7.2	6.7	1.47	346	13.2	81
	Water	3.6	3.2	2.9	81	7.5	6.9	1.47	378	13.2	81
II. Different types of baking powder	Tartrate	2.9	3.0	2.1	72	6.4	6.3	1.23	221	12.7	83
	Phosphate	2.9	2.8	2.2	75	6.7	7.0	1.11	244	13.5	86
	Sodium aluminum sulfate-phosphate										
	Brand No. 1	2.9	3.0	2.5	84	7.0	6.8	1.25	280	14.7	89
	2	2.9	3.1	2.6	89	7.0	7.0	1.18	274	14.6	86
	3	2.9	2.9	2.4	83	7.1	6.8	0.99	284	14.4	89
III. Different amounts of baking powder	Sodium aluminum sulfate-phosphate										
	Brand No. 3 12.3 g	3.0	2.9	2.6	86	7.0	6.9	1.03	263	13.7	84
	18.4 g	3.0	3.1	2.4	80	7.2	7.1	1.04	251	14.2	79
IV. Different proportions of bicarbonate of soda and baking powder, lactic acid being constant at 2.05 g	Soda 1.64 g, Baking powder 10.28 g	3.3	3.3	2.8	84	7.0	7.0	1.03	324	13.2	86
	Soda 2.50 g, Baking powder 6.54 g	3.3	3.2	2.3	70	7.2	7.5	1.03	326	12.5	80
	Soda 3.00 g, Baking powder 4.52 g	3.4	3.2	1.6	48	7.2	8.0	0.99	359	11.9	76
	Soda 3.50 g, Baking powder 2.16 g	3.4	3.2	1.0	30	7.3	8.1	1.04	399	12.1	70
	Soda 4.50 g, Baking powder 0.00 g	3.4	3.0	0.5	15	7.4	8.6	1.13	392	11.2	63

[1] Expressed as micrograms of thiamine per gram of dry matter.
[2] Calculations are based on the amount of thiamine found in the flour and in the milk.
[3] Expressed as percent of the calculated amount of thiamine in the dough.
[4] The displacement of the plunger for a stress of 28 g.
[5] Maximum score 100.

were from the same mill lot and were stored together until used. These differences are reflected in all of the values for the fresh milk biscuits in groups 1, 2, and 3. The only difference in ingredients for these biscuits was in the flour.

Retention of Thiamine. The thiamine retained in the doughs was practically 100% of that calculated, even in biscuits with excess of soda. For the biscuits made with the different liquids, the difference between the extreme values for retention of thiamine is not significant.[2]

The thiamine retained in the biscuits made with the phosphate and the tartrate powders was significantly less than that in the ones made with any of the sodium aluminum sulfate-phosphate baking powders. These results are the reverse of those reported by Williams and Fieger (1944), due perhaps to a lower and unvarying proportion of baking powder to flour in the biscuits of Williams and Fieger. With a 50% increase in the amount of sodium aluminum sulfate-phosphate baking powder, there was a significant drop in the retention of thiamine.

The thiamine retained in the biscuits decreased significantly with each increase in the amount of soda when the leavening was derived from a constant amount of lactic acid with equivalent and excessive amounts of baking soda.

pH of Dough and Biscuit. The pH of the doughs varied little except when tartrate or phosphate baking powder was used. In these doughs the pH values were significantly lower than in the others. It should be remembered that the difference between pH 7.0 and 7.5 is much less in terms of hydrogen ion concentration than is the difference between pH 6.7 and 7.0.

The greatest change in pH with baking occurred in the biscuits made with the different proportion of lactic acid and soda. With increasing amounts of soda, the pH of the baked biscuit increased progressively. This increase was 1.2 pH units with the largest amounts of soda.

Barackman (1942) states that ". . . most baking powders result in biscuits considerably above 7.0 pH." The results in Table III do not bear out this statement. All biscuits leavened with baking powder at the level recommended by the manufacturer were found to have a pH of 7.0 or below. Even with a 50% increase in the amount of baking powder of the sodium aluminum sulfate-phosphate type, the pH of the baked product was only 7.1.

pH and Thiamine Retention. From the figures in Table III, it will be seen that (except for the tartrate and phosphate baking powders)

[2] Throughout this discussion, differences are reported as significant if, using Student's "t" test, the odds are 99:1 or greater.

[3] Maximum score 100.

[4] Calculations are based on the amount of thiamine found in the flour and in the milk.

[5] Expressed as percent of the calculated amount of thiamine in the dough.

when the pH of the biscuit was close to 7.0, 80% or more of the thiamine was retained in the baked product.

Where the pH increased as the result of increase in the proportion of soda to acid ingredient or of increase in amount of baking powder, the retention of thiamine dropped. This agrees with results obtained on thiamine in pure solution (Beadle, Greenwood, and Kraybill, 1943), and with those of Barackman (1942). Farrer's (1945) experiments with thiamine in different salt solutions indicate that different buffer salts may influence the rate of thermal destruction of thiamine, and this may account for the lower retentions with the phosphate and tartrate baking powders. Melnick's results (1945) with thiamine in suspensions of baking powders are in agreement with this hypothesis.

Quality of Doughs. The different baking powders had a marked effect on the physical condition of the dough. With the tartrate powder, the dough was spongy and sticky; with the phosphate powder, the dough was somewhat spongy and sticky, but not as markedly so as with the tartrate powder. With the sodium aluminum sulfate-phosphate powders, the doughs were compact and were not sticky. The effect of these differences on external and internal appearance of the biscuits is shown in Figure 2. The doughs made with the different liquids exhibited only slight differences in physical condition and those made with the different amounts of soda and baking powder did not vary sufficiently to be detected during the manipulation.

Quality of Biscuits. The values for compressibility, breaking strength of the bottom crust, and height of four biscuits indicate that the flour from the different bags had more effect upon these factors than any other variable. It is pertinent, therefore, to discuss the differences in the values within groups rather than between groups. For the same reason, palatability scores will be considered only within groups.

The fresh milk biscuits in the first group were significantly more compressible than the others in the same group, which were similar to each other. There is no very significant difference between the breaking strength for the different types of biscuits. The fresh milk biscuits in this first group raised more than the others in the same group. The other biscuits were alike in their height.

The judges preferred the biscuits made with the whole milk. They found them more tender in crust and crumb and more pleasing in flavor than the other biscuits in this group. The total palatability score for the whole milk biscuits is significantly greater than that for any of the others in this group.

An increase of 50% in the amount of sodium aluminum sulfate-phosphate baking powder made no significant change in the compressi-

bility, breaking strength, and height of the biscuit. The judges always scored the biscuits with the extra powder as significantly inferior to those made with the regular amount. These biscuits were poorer in external and internal appearance, in flavor, and were less flaky than those made with less baking powder.

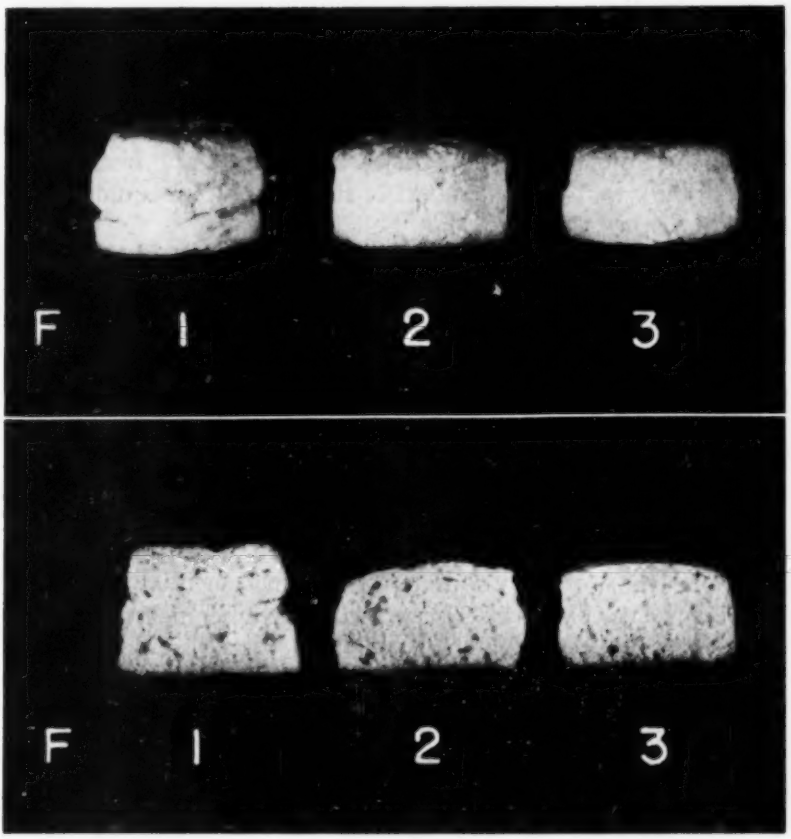


Fig. 2. External and internal appearance of biscuits made with different baking powders: 1. sodium aluminum sulfate-phosphate; 2. phosphate; 3. tartrate.

For the biscuits made with varying proportions of soda and baking powder, the compressibility did not vary with the changes in ingredients. The breaking strength, however, increased irregularly as the amount of soda was increased. The difference between the extreme values was significant, but was not significant between intermediate values. No explanation of this increase in toughness is offered. The height and the palatability of the biscuits decreased with increasing excess of soda to acid. The biscuits with soda at 3.00 g or more had a cream color, deepening with each increase in the amount of soda.

The judges were able to discern the flavor of soda at this and the higher levels. None of the biscuits were rated as unacceptable, however.

Summary

Biscuits were prepared using standardized formulae and preparation procedures. The effects of variation of ingredients on thiamine retention, pH, and quality of the biscuits were studied.

Biscuits made with fresh milk, evaporated milk, water, and dry whole milk plus water were alike in their thiamine content. The biscuits made with fresh whole milk retained slightly more of their thiamine than did the others. In eating quality, the biscuits made with fresh milk were judged superior; the others were equally acceptable to each other.

Biscuits made with sodium aluminum sulfate-phosphate baking powder (three brands) were alike in their thiamine content and retention. Increasing the amount of the baking powder by 50% (one brand) increased the loss of thiamine during baking. The biscuits made with a tartrate and with a phosphate baking powder lost more thiamine than those made with sodium aluminum sulfate-phosphate baking powders. All the biscuits were of similar eating quality.

Biscuits made with varying proportions of lactic acid, bicarbonate of soda, and baking powder retained less thiamine as the soda was increased. At the lowest level of soda 84%, and at the highest level of soda only 15%, of the thiamine was retained in the biscuits. The palatability of the biscuits decreased with each increment of soda, but none were inedible.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods. 4th ed. Lincoln, Neb.

Bailey, C. H.
1934 An automatic shortometer. Cereal Chem. **11**: 160–163.

Barackman, R. A.
1942 Thiamin retention in self-rising flour biscuits. Cereal Chem. **19**: 121–128.

Beadle, B. W., Greenwood, D. A., and Kraybill, H. R.
1943 Stability of thiamine to heat. I. Effect of pH and buffer salts in aqueous solutions. J. Biol. Chem. **149**: 339–347.

Conner, R. T., and Straub, G. J.
1941 Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem. (Anal. Ed.) **13**: 380–384.

Farrer, K. T. H.
1945 The thermal destruction of vitamin B_1. I. The influence of buffer salts on the rate of destruction of aneurin at 100°. Biochem. J. **39**: 128–132.

McKim, E., and Moss, H. V.
1943 Observations on the pH of chemically leavened products. Cereal Chem. **20**: 250–259.

Melnick, D., Oser, B. L., and Hochberg, M.
1945 Nutritional aspects of an improvement in baking powder formulation. J. Am. Dietet. Assoc. **21**: 611–615.

Platt, W., and Powers, R.
1940 Compressibility of bread crumb. Cereal Chem. **17**: 601–621.

Williams, V. R., and Fieger, E. A.
1944 Influence of ingredients on thiamine content of biscuits. Food Research **9**: 328–337.

REPORT OF THE 1945-46 METHODS OF ANALYSIS SUBCOMMITTEE ON THIAMINE ASSAY

AARON ARNOLD

Sterling-Winthrop Research Institute,
Rensselaer, New York

(Presented at the Annual Meeting, May 1946; received for publication May 23, 1946)

The rapid methods for vitamin determination have come into favor among mill chemists who are faced with checking the enrichment level in flour during production. Towards evaluating and unifying current practice in the rapid methods for thiamine, a collaborative study was undertaken to compare the salient features of the published methods.

The rapid assay methods for thiamine in flour save time by eliminating the enzyme digestion and the base exchange steps of the A.A.C.C. thiamine method (*Cereal Laboratory Methods*, 1941; Andrews, 1944). This may be done in the analysis of flour since only a small fraction of the thiamine occurs bound as the pyrophosphate; on the other hand, the rapid methods for bread (Schiller, 1944) require the enzyme digestion step because of the appreciable content of bound vitamin.

Nicholls *et al.* (1942) and Glick (1944) successfully used dilute acid at room temperature to extract thiamine from flour. In contrast Andrews and Nordgren (1941), who first published a rapid thiamine method for flour, and Hoffer, Alcock, and Geddes (1943) observed acid potassium chloride solution to be an effective medium for extracting the vitamin. Although the latter two groups of investigators both used the same extracting solution, they differed as to the temperature at which the extraction was carried out; Andrews and Nordgren (1941) obtained satisfactory vitamin recovery at room temperature, while Hoffer and associates recommended extraction at elevated temperatures. Finally, Glick, and Andrews and Nordgren favored clarification of the extract by filtration, while Nicholls *et al.* and Hoffer and co-workers observed that extract clarification by centrifugation gave equally satisfactory thiamine extraction and was more rapid.

The points in the foregoing methods which appeared to require checking were (a) the type of extracting medium, (b) the temperature of extraction, and (c) the method of clarifying the extract. The procedure for the alkaline ferricyanide oxidation of thiamine to thiochrome is probably sufficiently well standardized so as not to require collaborative study.

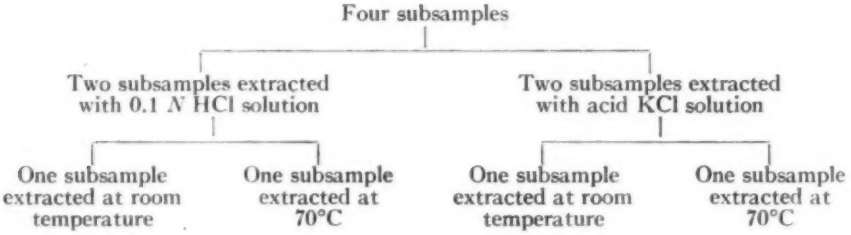
With these points in mind, as well as the limitations of the amount of work which may be expected in collaborative study, the following

paired procedure-steps were compared: 1. extraction with dilute acid vs. acid potassium chloride solution; 2. extraction at room temperature vs. elevated temperature; and 3. extract clarification by filtration vs. centrifugation.

While conclusions drawn from collaborative study are less subject to error when several samples are studied, only one sample was used in the present study in view of the several alternative points of procedure which were to be investigated.

Experimental

The collaborators were requested to analyze an A.A.C.C. National Check Sample of flour,[1] and also this sample to which was added 2 μg of thiamine per gram of sample [2] by the following variations in procedure:


Four subsamples
Two subsamples extracted with 0.1 N HCl solution
Two subsamples extracted with acid KCl solution
One subsample extracted at room temperature
One subsample extracted at 70°C
One subsample extracted at room temperature
One subsample extracted at 70°C

The directions supplied for conducting the analyses were essentially as follows:

Place the selected quantity of flour (2.0 g is suggested for the present study) in a 125 or 250 ml Erlenmeyer flask, and add 50 ml of 0.1 *N* hydrochloric acid or 50 ml of 25% potassium chloride solution acidified with 2% acetic acid or 0.1 *N* hydrochloric acid. Shake the uniformly suspended sample for 30 minutes in an automatic shaking device or intermittently by hand (incubate four of the portions at room temperature and the remaining four at 70°C). At the end of this time centrifuge the samples until the supernatant liquid contains only a negligible amount of suspended matter or filter them (pour the first portion of the filtrate back through the filter).

For the oxidation of thiamine to thiochrome, rapidly add 3 ml of 15% sodium hydroxide solution to a 5 ml aliquot of the clarified extract in a centrifuge, separatory funnel, or equivalent device, mix the contents, add 2 drops of 1% potassium ferricyanide solution, and again

[1] Samples prepared for mailing were supplied by W. R. Urban, Chairman of the A.A.C.C. Check Sample Committee. This was most useful, further, in that the collaborators were not required to test the sample by the A.A.C.C. procedure since the Check Sample value was already at hand.

[2] To reduce the possibility of differences developing owing to variations in the amount of thiamine added, ampules of thiamine hydrochloride solution at a concentration of 0.5 mg/ml were provided. It was directed that after appropriate dilution with either dilute acid for the one series or with acid potassium chloride solution for the second, a portion of the diluted thiamine hydrochloride solution be compared with the collaborator's own thiamine standard.

mix rapidly and uniformly. After 1 minute add 13 to 16 ml of isobutanol (the exact volume being determined by the routine practice in the collaborator's laboratory), shake the contents for 1 minute, centrifuge, and draw off the water layer. Clarify the alcohol layer by adding 1 ml of ethanol and estimate the fluorescence in the usual way. The attention of the collaborators was called to the fact that some time is saved by storing the isobutanol extracts in the cuvettes until the full series is at hand for reading (see Andrews, 1944).

Express the results in μg of thiamine per gram of sample.

Results and Discussion

The results of the study, in which 16 laboratories participated, are summarized in Table I. At the outset it may be stated that the results of the study are in accord with the conclusions of Hoffer, Alcock, and

TABLE I

SUMMARY OF RESULTS OF THE THIAMINE CONTENT OF A COLLABORATIVE FLOUR SAMPLE AND OF THE SAMPLE WITH 2 μG OF ADDED THIAMINE BY SEVERAL MODIFICATIONS OF A RAPID METHOD

Extraction medium	Extraction Temp.	Method of extract clarification: Centrifugation (9 collaborators) — No. of det'ns.	Thiamine content[1,2] μg/g — Range	Mean[3] ± S.E.	Combined mean ± S.E.	Filtration (8 collaborators) — No. of det'ns.	Thiamine content[1,3] μg/g — Range	Mean[3] ± S.E.	Combined mean ± S.E.
COLLABORATIVE FLOUR SAMPLE									
Dilute acid	R.T.	19	3.37–4.34	3.71 ± .06	3.74 ± .04	20	3.07–4.01	3.59 ± .05	3.63 ± .04
	70°C	19	3.33–4.21	3.78 ± .06		19	3.07–4.19	3.67 ± .07	
Acid KCl	R.T.	19	3.63–4.41	3.92 ± .06	3.96 ± .04	22	3.52–4.38	3.81 ± .06	3.88 ± .04
	70°C	19	3.68–4.57	4.00 ± .07		19	3.61–4.59	3.96 ± .06	
COLLABORATIVE FLOUR SAMPLE WITH ADDED THIAMINE, 2 μG/G FLOUR									
Dilute acid	R.T.	19	5.22–6.14	5.52 ± .08	5.60 ± .05	20	5.28–6.35	5.56 ± .07	5.60 ± .05
	70°C	19	5.20–6.14	5.68 ± .07		19	5.14–6.35	5.64 ± .08	
Acid KCl	R.T.	19	5.00–6.97	5.99 ± .1	6.07 ± .07	19	5.43–6.58	6.00 ± .08	6.04 ± .06
	70°C	20	4.75–7.04	6.15 ± .09		19	5.54–6.85	6.07 ± .08	

[1] The thiamine content of the low-protein flour sample used in this study was approximately 4.12 μg/g, the mean reported by the Check Sample Service Committee.

[2] These thiamine values were calculated in terms of an external thiamine standard.

[3] The means were obtained by averaging the individual determinations submitted by the collaborators. The results of one collaborator were increased by 10% to compensate for a difference in the standards.

Geddes (1943). As reviewed above, these investigators recommended sample extraction with acid potassium chloride solution at elevated temperature followed by centrifugation of the extract. The discussion in the following paragraphs gives the reasons for these conclusions.

Extraction Medium. There are data on eight pairs of samples in the table, two at room temperature and two at 70°C for the extracts clarified by centrifugation, and a like number for the extracts clarified by filtration. These data may be used to compare the completeness of extraction by dilute acid with that resulting from the use of acid potassium chloride solution. The combined means clearly show more thiamine was extracted by acid potassium chloride solution than by dilute acid in every instance. Comparable pairs differ only 6 to 8% but the results are consistent enough that statistical analysis shows that a difference of this magnitude would not occur by chance as often as once in a thousand trials. The data are thus interpreted as indicating that acid salt solution is the extraction medium of choice.

One of the collaborators reported that "the standard made up in acid potassium chloride solution apparently deteriorated so rapidly that no reading was obtained. The standard made up with 0.1 *N* hydrochloric acid, however, gave the expected results." The uncommon and unexplained difficulty encountered by this collaborator has not been reported by others.

Temperature of Extraction. The data in Table I yield eight pairs of analysis bearing on the comparison of the two temperature conditions for extraction. As in the comparison of the two extraction media, in every instance slightly more thiamine was extracted at elevated temperature than at room temperature.

The individual pairs do not differ significantly, however, nor is a statistically significant difference obtained when all the results bearing on this factor are combined into a single difference. When this is done, only 3% more thiamine appears to have been extracted at 70°C than at room temperature. Since chance alone was not likely to cause slightly better thiamine extraction at the higher temperature in a comparison of eight pairs, the results are interpreted to indicate that extraction at 70° C is to be preferred to extraction at room temperature.

One of the collaborators (J. H. Lanning) reported that extraction at room temperature is favored in his laboratory. This is done to avoid the possible slight errors which may result when aliquots of solutions at a temperature appreciably different from 20°C are measured out. Changes in volumes due to evaporation are also avoided. In view of the slightly better vitamin extraction at the higher temperature it is possible that errors are more than compensated for when due to the foregoing reasons.

Method of Extract Clarification. The data given in the table likewise bear on the problem of the method of clarification of the extracts. Fortunately, there was an even division among the collaborators, eight utilizing filtration to clarify the extracts and nine utilizing centrifugation. The results of six of the eight pairs show slightly better thiamine extraction when the extracts were clarified by centrifuging. Only two of the results show better thiamine extraction when the extracts were clarified by filtering them. Considering that the object of the rapid method is to save time, these data strongly support clarification of the extracts by centrifugation. Using the centrifuge not only saves considerable time but also gives, on the average, a result slightly nearer that obtained by the A.A.C.C. method.

Several collaborators mentioned that extraction with acid potassium chloride solution yielded clearer extracts by centrifugation than did dilute acid extracts. The foregoing preferred procedures thus complement each other.

Thiamine Recoveries from the Samples with Added Thiamine. To determine the effect of the procedures on added vitamin, 2 μg of thiamine were added to portions of the sample by the collaborators. When acid potassium chloride solution was the extracting medium, slightly more thiamine than this appears to have been recovered. As judged by the combined means, the recovery was 2.13 to 2.16 μg of thiamine. This is simply taken to mean that slightly more of the thiamine in the sample itself was extracted from these portions since it was theoretically possible to obtain as much as 6.12 μg of thiamine from these samples (2 μg of thiamine added to a sample which was reported to contain 4.12 μg of thiamine per gram). The use of dilute acid as the extracting medium served to extract most of the added thiamine though not all; 1.86 to 1.97 μg of added thiamine recovered as judged by the combined means.

The over-all results do not favor the use of an internal thiamine standard, i.e., calculation of the thiamine content of the sample based on the fluorescence of the 2 μg thiamine increment added to a second portion of the sample, as a means of evaluating the thiamine content of the sample. Under conditions of good extraction, calculations of the thiamine content of the sample based on an external standard appear to yield reliable values. The slight upward correction factor recommended by Hoffer *et al.* (1943) corrects for the bound thiamine in the sample rather than for incomplete extraction of the free thiamine. This correction factor is somewhat dependent on the type of flour studied.

Oxidation Procedure. For the study, alcohol clarification of the isobutanol extract was suggested rather than the more usual clarifica-

tion with anhydrous sodium sulfate. Recently Watson (1946) has also recommended alcohol clarification to avoid the possibility of fluorescence inaccuracies attributable to the anhydrous sodium sulfate. Several collaborators observed alcohol clarification to be unsatisfactory so that it did not meet with universal approval. With care the two procedures may probably be used interchangeably.

Similarly, the procedure as given followed Andrews' (1944) recommendations, recently confirmed by Watson (1946), regarding the successive addition of alkali and ferricyanide for the oxidation of thiamine to thiochrome. Collaborator objection was voiced regarding the use of these reagents separately rather than together. The use of these reagents separately does not prove burdensome once the procedure is established and is to be recommended on the basis that it yields results which appear to agree better with expected values.

One collaborator (D. Melnick) has mentioned that the use of an automatic pipette shaker, of a type on the market, to rock the separatory centrifuge funnels during the oxidation step eliminated the troublesome factor of lack of uniformity because of shaking. Two funnels are readily shaken at a time by constructing a simple adapter for the shaker.

Summary

On the basis of the means of the results of the 1945–46 collaborative study, in which 16 laboratories participated, the following conclusions are drawn:

1. Extraction with acid potassium chloride solution appears to extract significantly more thiamine than does extraction with dilute acid. 2. Extraction at 70°C appears to be slightly more effective than room temperature for extracting thiamine from flour samples. 3. Extract clarification is accomplished more rapidly and with at least as good thiamine recovery by centrifugation as by filtration. 4. Extraction of added thiamine was complete by the preferred procedures so that confirmation of their effectiveness, as judged by study with the particular sample submitted to the collaborators, was obtained.

Acknowledgments

The writer wishes to express his appreciation to Mrs. Mary Lenz for the statistical treatment of the data and to the following who participated in the collaborative study: A. W. Alcock, Purity Flour Mills, Winnipeg, Manitoba; H. J. Alleman, The Kroger Food Foundation, Cincinnati, Ohio; H. M. Boyd, General Mills, Minneapolis, Minnesota; L. J. Daniel, Cornell University, Ithaca, New York; C. F. Davis, Schwarz Laboratories, New York, N. Y.; W. F. Geddes, University of Minnesota, St. Paul, Minnesota; R. L. Gray, Novadel-Agene Corporation, Buffalo, New York; L. W. Haas, The W. E. Long Company, Chicago, Illinois; C. Hoffman, Ward Baking Company, New York, N. Y.; A. J. King, Fisher Flouring Mills Company, Seattle, Washington; J. H. Lanning, Continental Baking Co., Jamaica, New York; E. Lerner, Lab-

oratory of Vitamin Technology, Chicago, Illinois; D. Melnick, Food Research Laboratories, Long Island City, New York; M. D. Mize, Wallace and Tiernan Company, Chicago, Illinois; J. H. Panton, Winthrop Chemical Company, Rensselaer, New York; W. R. Urban, Omaha Grain Exchange Laboratories, Omaha, Nebraska; W. H. Ziemke, Quality Bakers of America, New York, N. Y.

Literature Cited

American Association of Cereal Chemists
1941 Cereal Laboratory Methods. 4th ed.

Andrews, J. S.
1944 Report of the 1943–44 methods of analysis subcommittee on thiamine assay. Cereal Chem. **21**: 388–397.

——, and Nordgren, R.
1941 The application of the thiochrome method to the thiamin analysis of cereals and cereal products. Cereal Chem. **18**: 686–695.

Glick, D.
1944 Simplified procedures for the determination of thiamine in wheat flours and bread by the thiochrome method. Cereal Chem. **21**: 119–126.

Hoffer, A., Alcock, A. W., and Geddes, W. F.
1943 A rapid method for the determination of thiamine in wheat and flour. Cereal Chem. **20**: 717–729.

Nicholls, J. R., Booth, R. G., Kent-Jones, D. W., Amos, A. J., and Ward, H. H.
1942 Vitamin B_1 (aneurine) assay in white flour. Analyst **67**: 15–17.

Schiller, G. W.
1944 Simplification of the thiochrome method for thiamine determination. Cereal Chem. **21**: 544–548.

Watson, H. A.
1946 Study of some factors influencing the oxidation of thiamine to thiochrome. Cereal Chem. **23**: 166–177.

BOOK REVIEWS

Hackh's Chemical Dictionary. By Julius Grant. 925 pp. The Blakiston Company, Philadelphia, Pa. 1946. Price $8.50.

The third edition of this well-known and authoritative chemical dictionary was published in 1944 and reprinted in 1946 with some changes and additions. It contains some 57,000 entries and not only covers the terms used in chemistry and a brief statement of chemical theories and laws but also includes the vocabularies of physics, geology, mineralogy, botany, zoology, medicine, and pharmacy. Many of the terms used in industry, mining, and commerce are included.

The properties of the elements and a large number of compounds are described. Compounds are listed and described in systematic order, including the name, formula, molecular weight, synonyms, occurrence, preparation or type of substance, appearance, physical properties, as well as chemical, industrial, and medical uses. Various types of chemical apparatus are listed and brief biographies with portraits are given of scientists who have made important contributions to the progress of chemistry.

This book contains such a large body of classified information in the field of chemistry and related sciences that it will prove to be a valuable addition to any chemist's library.

W. F. GEDDES
Division of Agricultural Biochemistry
University of Minnesota
St. Paul 1, Minnesota

Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Sixth Edition. 932 + xii pages, 76 illustrations, and 31 reference tables. Published by the A.O.A.C., Box 540, Benjamin Franklin Station, Washington 4, D. C. 1945. Price per copy domestic $6.25, foreign $6.75. ($5.00 to members when ordered through the office of the Association.) If five or more copies are ordered at one time for delivery to a single address, the price per copy is $5.00 domestic and $5.40 foreign.

The most recent revision of this compilation of methods again offers to analysts a wealth of information on procedures for use with agricultural, food, and drug prod-

ucts. Most of the material of the fifth edition has been retained, and to it substantial additions have been made—as witness the increase of nearly 200 pages over the previous edition. Five new chapters have been added—on cosmetics, enzymes, gelatin products, vitamins, and extraneous materials in foods and drugs.

Of the new chapters, those on vitamins and on extraneous materials represent the most significant additions and will be of chief interest to the cereal chemist. Included in the former chapter are familiar techniques for thiamine and riboflavin, as well as procedures for the estimation of vitamin A and carotene, for niacin (by microbiological means), and for vitamins D and K. The chapter on extraneous materials will be welcomed by all food and drug processors, embodying as it does a number of tested procedures useful in maintaining a high standard of plant sanitation.

Cross references again abound—a style which is doubtless economical of space, but which certainly does not facilitate ready use. It is still usually necessary in looking up an unfamiliar method to assemble the complete information from at least two, and often more, isolated paragraphs, and to add whatever operating notes may be necessary for best results. Brevity of presentation is often commendable, but on other occasions may lead to some difficulty. Thus in the determination of DDT by the labile chloride method, no mention is made of the fact that presence of chlorides from any other source will invalidate results. This fact will be apparent to the thoughtful analyst who considers the chemical basis of the procedure, but is not readily evident from the text. Other similar examples are numerous.

Printing and binding are well done, and the book appears reasonably free from typographical errors. Again this treatise will find a welcome place on the reference shelves of most cereal laboratories.

F. C. HILDEBRAND
General Mills, Inc.
Minneapolis, Minnesota

The Baking Industry. By Mary Kinnavey Moore. 32 pp. Billman Publishing Co., Inc., Boston, Mass. 1946. Price $1.00.

The baking industry, the country's largest food industry in number of employees and payroll size and the world's oldest manufacturing occupation, has followed closely the advancement of agriculture. Starting with home baking and following the trend established by industrial revolution, baking expanded into neighborhood shops and then into large wholesalers. Statistics are quoted as to the dollar value of the industry and as to the consumption of flour per capita.

The classifications of the baking industry are given in detail—wholesale, retail, and house-to-house operations.

The subdivisions of baking are thoroughly explained, from bread production through sales.

Anyone in the baking industry will be interested in Part IV on personnel. This is a very good outline of the duties of each person employed in the baking business.

Statistics are given as to wages earned by those employed in this industry; 201,533 employees earned $262,001,562 per year. Workers can choose between the handcraft production in small shops or machine production of large plants, and occupational hazards are few.

This is a good review of the baking industry.

W. A. RICHARDS
Russell-Miller Milling Company
Minneapolis, Minnesota

"Speak for yourself, John..." and that's what we're going to do!

Product research and development by
Special Markets Division, Winthrop Chemical Company, Inc.
in the interest of the Cereal Chemistry Industry.

FIRSTS by Winthrop in the Cereal Chemistry Industry

FIRST to provide ADEQUATE POTENCY

FIRST to establish A STANDARD OF COMPARISON for free-flowing properties of flour-enrichment mixtures

"B-E-T-S"—*did the job FIRST*—provided a formula that adequately enriched bread to government requirements, regardless of grade of flour used! The industry's adoption of a minimum enrichment addition, to assure compliance with government standards, is evidence of the soundness of this policy.

WINTHROP technicians recognized early in the flour-enrichment program that "free-flowing" was one of the prime requisites for a superior flour-enrichment mixture. Their success in developing a workable formula embodying this property is evidenced in "VEXTRAM", which is recognized as the standard of comparison. "VEXTRAM'S" *starch base and other carriers add practically no mineral ash to flour!*

WINTHROP'S RIBOFLAVIN MIXTURE for feed is a free-flowing concentrate designed to maintain high riboflavin content in mixed feeds. Every ounce provides 1 gram —1,000,000 micrograms—of riboflavin.

Stocked for quick delivery at New York, Chicago, Kansas City (Mo.), Denver, San Francisco, Portland (Ore.), Dallas and Atlanta.

OTHER WINTHROP NUTRIENTS

Crystalline **VITAMIN B1** — *Crystalline* **VITAMIN C**

CALCIUM PANTOTHENATE

RIBOFLAVIN — **VITAMIN B6**

CALCIFEROL — **TRIDEE** — **AMINO ACIDS**

WINTHROP

Address Inquiries to—

Special Markets Division

WINTHROP CHEMICAL COMPANY, INC.

170 Varick Street, New York 13, N. Y.

You Can Depend Upon These All-Vegetable Hydrogenated Shortenings . . . .

PRIMEX B&C

An all-hydrogenated vegetable oil shortening of exceptional stability. Excellent for all deep frying purposes. Preferred by biscuit and cracker bakers, manufacturers of prepared biscuit, pie crust, and doughnut flours, and makers of other food products where rancidity troubles are to be avoided.

SWEETEX

The "High-Ratio" shortening. Especially designed to permit bakers to produce "High-Ratio" cakes, icings, and sweet yeast goods with superior eating and keeping qualities.

PRIMEX

The all-hydrogenated shortening "that sets the quality standard." A top grade shortening especially recommended for doughnut frying, for pies, cookies and bread, and for other shortening purposes.

PROCTER & GAMBLE

Branches and warehouses in principal cities

General Offices . . . CINCINNATI, OHIO

CONTROL

UNBIASED laboratory tests over a period of several years have conclusively proved that NATIONAL GRAIN YEAST is far superior in the matter of dough control.

And it is because of this and other outstanding qualities that thousands of progressive bakers have come to regard NATIONAL GRAIN YEAST as a primary essential in the art of baking better bread.

NATIONAL GRAIN YEAST CORPORATION

Chanin Bldg., N. Y. C. • Chicago, Ill. • Crystal Lake, Ill. • Belleville, N. J

Frank J. Hale
President

A COMPLETE PRINTING SERVICE

GOOD PRINTING does not just happen; it is the result of careful planning. The knowledge of our craftsmen, who for many years have been handling details of composition, printing and binding, is at your disposal. For over sixty years we have been printers of scientific and technical journals, books, theses, dissertations and works in foreign languages. Consult us about your next job.

• PRINTERS OF • CEREAL CHEMISTRY

LANCASTER PRESS, Inc.

PRINTERS • BINDERS • ELECTROTYPERS

ESTABLISHED 1877 LANCASTER, PA.

WRITING SYMPOSIUM REPORTS

SPECIAL REPRINT CONTAINING THE FOLLOWING ARTICLES

"They Understand Not One Another's Speech"
"Short Cuts to Unity, Clearness, and Brevity"
"Role of Statistics in Technical Papers"
"Preparation of Illustrations and Tables"
"Preparation of Technical Reports"
"Preparation of Papers for Oral Presentation"

Originally presented at the Thirtieth Annual Meeting of the A.A.C.C., Minneapolis, Minnesota, 1944

Reprinted from *Transactions*, Vol. III, No. 2

50 cents a copy

Write to Cereal Chemistry, University Farm, St. Paul 8, Minnesota

AMERICAN ASSOCIATION OF CEREAL CHEMISTS

Photelometer

CENCO - SHEARD - SANFORD ★

More Accurate—More Rapid—More Reliable Than a Colorimeter

No. 12335A

PHOTELOMETRIC methods of analysis replace many of the usual colorimetric methods with slight modifications in procedure. No comparison standards are needed. A single calibration for any particular unknown holds indefinitely. Such simplification in laboratory practice greatly facilitates the work and saves much time in important analyses. In the industrial laboratory, photelometric methods are being used for the determination of molybdenum, vanadium, cobalt, manganese, copper, chromium, iron, phosphorus, aluminum, ammonia, calcium, chlorine, fluorine, magnesium, nitrates, nitrites and sulphates in water; and in innumerable other analyses.

Write for full information. Ask for Bulletin No. 104A

No. 12335A for 115 volts—60 cycles A.C.
(Controlled frequency). Each..... **$260.00**

CENTRAL SCIENTIFIC COMPANY

SCIENTIFIC INSTRUMENTS CENCO LABORATORY APPARATUS

NEW YORK TORONTO **CHICAGO** BOSTON SAN FRANCISCO

WHAT LEAVENING?

The biggest question behind a prepared mix . . . . . .

HOW TO ANSWER IT..

Of all the ingredients that go into the making of a prepared mix, none presents a more complex problem than the leavening.

Upon the leavening largely depends the shelf life of the mix as well as the volume, lightness, grain, and color of the oven product.

The problem is further complicated by the fact that a leavening found most efficient for a particular gingerbread may not prove at all satisfactory for a waffle, muffin, cookie, or other type of mix. *The leavening must be tailor-made for the specific mix in question.*

Importance of Rate Reaction

Every leavening acid has its own characteristic rate of reaction with soda. Rate of reaction is, therefore, a primary factor to consider. Excessive loss of leavening gas while mixing the batter or dough must be avoided. The leavening should provide sustained action throughout the oven period without "tailing off" before the end of the bake.

How Much Leavening?

Then there is also the question of *quantity* of leavening. Sufficient leavening should be included in the mix to provide maximum expansion in the oven with a margin of safety to assure adequate shelf-life. Too much leavening must be avoided or the cake may blow-up in the oven.

Today, better than 95% of all chemical leavening includes a phosphate or phosphates combined in varying proportions. The question is which phosphate or phosphates . . . and how much?

For over forty years Victor Chemical Works has specialized in the production of food phosphates. From Victor's Research Laboratories have come many of the outstanding developments in the science of chemical leavening.

Get the Answer From Victor

If you would like help in answering the all-important leavening question, our laboratory will gladly submit suggestions for the most efficient leavening.

VICTOR PHOSPHATES

For Leavening

Monocalcium Phosphate
"Regent"—(hydrated)
"V–90"—(anhydrous)
Sodium Acid Pyrophosphate
"Victor Cream"
"Perfection"

For Mineralization

Monocalcium Phosphate
Dicalcium Phosphate
Tricalcium Phosphate
Calcium Pyrophosphate
Sodium Iron Pyrophosphate
Iron Orthophosphate
Iron Pyrophosphate

. . .

Experimental samples on request

VICTOR CHEMICAL WORKS

WORLD'S LARGEST MANUFACTURERS OF FOOD PHOSPHATES

141 West Jackson Boulevard Chicago 4, Illinois

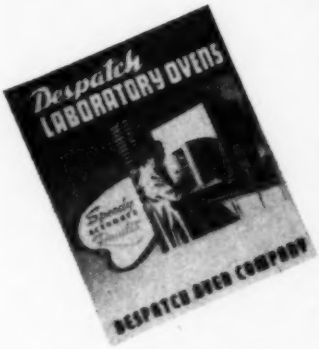
GET FAST, CONTROLLED DRYING

for Flax and Soy Tests

with

DESPATCH BUILT

LABORATORY OVENS



***WRITE TODAY** for this helpful new Bulletin 105-CC describing Despatch Laboratory Ovens. Fully illustrated; printed in 2 colors.*

There's no faster, safer, more accurate method of drying flax or soy samples than to run them through a forced-draft *Despatch* Laboratory Oven! Every test gets optimum heat application, accurately controlled.

You can run *oil content determinations* (drying before grinding and re-drying to eliminate solvent), *moisture content tests, constant weight analyses* and scores of other tests with equal ease and accuracy.

FEATURES: Fast heat recovery, 2–8 min. after loading cold samples • Thermostat sensitive to ±1°C. • Wide-range flexibility, 35° to 260°C. • 5 year guarantee of heat elements • Convenient sizes, 13" x 13" x 13" to 37" x 25" x 37"; electric or gas models.

Ask your Dealer for Details

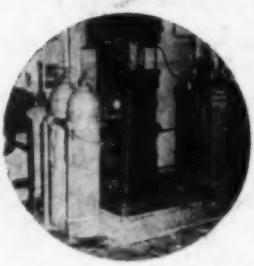
or write direct

DESPATCH OVEN COMPANY MINNEAPOLIS 14 MINNESOTA, U.S.A.

North, South, East or West - - - -

N-A's nationwide flour treatment service means — prompt shipment from strategically located N-A warehouses, quick response to service calls by N-A Servicemen, assistance on maturing, bleaching and enriching problems and uniform flour treatment results with —

AGENE **NOVADELOX** **N-RICHMENT-A**



WALLACE & TIERNAN CO., INC., Agents for:

NOVADEL-AGENE

N-A 131 BELLEVILLE, NEW JERSEY